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Can high energy physics be too easy?

THE Harvard physicist Sheldon Glashow, who with 'BJ' Bjorken invented charm, remarked to a visiting *Nature* reporter last week that we were in danger of losing a whole generation of high energy physicists. Not, for once, for lack of jobs or money but because students were turning to mere calculation, and not tackling the 'real problems' of physics. His students, said Glashow, want to play with the equations of 'gauge theory', developing new solutions and thus investigating the properties of the equations, rather than investigating the properties of matter. Gauge theories appear to have made life too easy. But what exactly are the 'real problems' that the students should tackle?

Gauge theories, with a useful addendum known as 'spontaneous symmetry breaking', have become the predominant theory of particle physics in the last few years. By them, most of the threads of the subject can be hustled into a fairly respectable shape, with only a few loose ends. The loose ends can encompass a great deal. For example, one of the major problems on which physicists cracked their heads in the 60s—what happens when one hadron, such as a proton, hits another—can now be seen as little more than a technicality. The complications are caused by throwing a bunch of quarks at another bunch, rather than single quarks at single quarks.

The quark-quark interaction seems now to be explicable by the gauge theory known as 'quantum chromodynamics' (QCD); the rest of hadron dynamics is complication. Even confinement—the means by which quarks are retained in the hadron—may be seen as an effect ultimately derivable from QCD. It may be that high energy physics is spawning a sub-group of 'hadron physicists', who will be left to deal with the complications of hadrons, just as the subject spawned nuclear physicists at an earlier date. In this view, the 'real physics' comes in explaining the properties of the quarks (and the leptons, like the electron and the neutrino, which come with them).

For Glashow, the deep problem is why nature chooses to repeat itself. The everyday, low energy world can be explained with two quarks (up and down) and two leptons (electron and electron neutrino). But then comes another set—more massive, but mirroring the properties of the first. These are the strange and charmed quarks, the muon and muon neutrino. Experiment appears to be revealing a third set, and there seems little reason why there should not be more. Why this repetition? Glashow has been working on it, but has been unable to make much progress. He has turned to the history of physics to see if such a problem has faced physics before; he has concluded that it is unique.

For Steven Weinberg, one of the creators of the gauge theory paradigm and himself a Harvard physicist,

the great challenge is to incorporate gravity into the gauge theory unification of forces. Judging by the activity of the CalTech physicist Murray Gell Mann, he agrees with Weinberg. Both have been working on 'supersymmetry', the invention of CERN theorists, which offers hope of unification. But, Weinberg told our reporter, two years of great technical progress have brought little contact with experiment.

On the experimental side, the next great leap will probably be the testing of the gauge theories, and the discovery of precisely which gauge model fits nature. Many experimenters are by nature iconoclasts and will be happiest if they defeat gauge theory; but the majority expect it to survive. The experiments will probably reveal more quarks and leptons, and the plethora of particles that gauge theory predicts: intermediate vector bosons, Higgs particles and the like. And perhaps the unexpected that will lead us forward.

Experiment is ultimately the source of all physical knowledge, but among theorists Glashow's students, and others interested in the apparently purely mathematical properties of gauge theories, may yet be tackling the 'real problems'. In the past, mathematical juggling yielded Lagrangian and Hamiltonian mechanics from Newton's theory; both were a necessary precursor to an understanding of quantum mechanics. Newton had no concept of energy, nor of the principle of least action, both extremely fruitful concepts which derive from the original Newtonian equations.

Rutherford is said to have remarked "one should no more let the mathematicians take over physics than let the military take over power". But there should be an exception to his maxim: when the mathematicians are creating new physical concepts with the equations at their disposal.

Many of the properties of gauge theories can only be investigated in the 'classical' approximation—that is, ignoring quantum effects and treating the equations just like those of Maxwell's equations of electromagnetism; quantisation comes later. This is attractive to students, who find themselves facing problems not unlike the ones they solved as undergraduates, with the exciting difference that they are at the frontier of physics. The calculations are very sophisticated, and require the development of great skills and ingenuity. Should they be discouraged?

Particle physics has been moving too fast; it needs time for reflection. Gauge theories with spontaneous symmetry breaking are strange mixtures of ideas; it is not at all clear that their significance has been thoroughly absorbed yet, or that they have been cast in anything like their most illuminating form. So let the mathematicians play—provided that ultimately, they come up not with mathematics, but with ideas. □



Collaboration with the first world can be fruitful

Dr P. T. Haskell, Director of the Centre for Overseas Pest Research (COPR), London, argues for close cooperation in science between the developing and industrialised world.

THE article on research in third world countries (2 March, page 8) presents a gloomy picture of non-cooperation between industrialised and developing countries. But the charges it reports of "scientific colonialism" may be more misleading than helpful.

Of course "scientific colonialism" occurs, but there is no evidence to show that it is widespread in first/third world projects. Much depends on the presentation of the objectives of the work so for countries to claim that first world researchers exploit "lax safety and health regulations" is meaningless unless actual cases are cited. My laboratory has projects in several developing countries on the long term environmental effects of insecticides and this work has, in some instances, necessitated the application of DDT, an insecticide now banned in most of the first world. Should I therefore be accused of "colonialism" or of "exploiting lax safety regulations" by providing developing countries with information on this compound relevant to the improvement of their agriculture and public health?

Similarly the unqualified statement that "third world scientists involved are left untrained . . . and the visiting team . . . takes its data with it" is simply untrue in a large number of cases for which I can give chapter and verse. Again, although I agree that it has occurred, evidence is required on the nature and extent to support such a sweeping generalisation and to clarify the root causes. Nowadays research contracts with developing countries usually contain clauses about publication of results and retention of data. One of the problems, however, is that developing countries often do not possess the facilities or resources to store or use large bodies of data; unless some special arrangement is made this is lost to science and may result in the later duplication of work.

The claim that because of funding pressure "the North is making a *de facto* determination of the science policy of the South" needs to be looked at more critically. There have been many cases of the attempted transfer of inappropriate western technology into developing countries. The main reason for this, however, is not funding pressure, but the cultural gap between scientists and science policy administrators in both the industrialised and developing countries.

There is certainly considerable scientific arrogance in the first world about the value of its scientific solutions, often joined to ignorance about the relevance of developing country practice. This has been nowhere more apparent than in agricultural development, where, for example, only relatively recently has there been recognition of the virtues of traditional peasant agricultural systems and the need to conserve certain aspects of them and support them with appropriate modern technology. To do this, however, needs considerable effort to increase communication between, in this example, farmers and scientists, and it is often the lack of this basic exchange of ideas which impedes and corrodes the relationship between first and third world science.

Let me now turn to the contention that one of the objectives of research collaboration is to seduce the best brains from developing countries to work in and for the first world. The implication is that interchange of scientists should be stopped. This certainly runs contrary to the views expressed to me on many occasions in developing countries that one of the best positive aspects of first/third world science collaboration is the training and experience which

developing-country scientists can derive from a spell of work in an industrialised country.

It is a fact that a proportion of such visitors stay and take up employment; but what are the reasons for this? In every case known to me there are two important ones. The first is that the scientist is in a field where the facilities available in his home country simply do not allow him to conduct the research he wants to carry out. The second is economic: at home there are no jobs or the rate of pay is too low. The latter is important. It concerns science policy in that support for research and the necessary institutions often has a low priority in many developing countries.

I was closely concerned for many years with the (UNDP/FAO) Desert Locust Project. During the course of the project more than a hundred developing country scientists were given advanced training. A survey carried out at the end of the project showed that less than a third of those trained were working at the job for which they were trained. Many had gone to commercial companies because of better pay; several found their training allowed them to apply for jobs as science administrators in departments other than the one which requested the original training; others discovered that there were simply no posts in their discipline in government service. It could be argued that this represents a loss of talent in a particular sphere of science in the developing countries concerned; or it could be argued that the total country stock of scientists of a particular level had been increased. The difficulty is to decide what disposition of these scientists will give maximum benefit to their home country—and that is a matter for the science policy of that country.

The last point I want to comment on is the very curious claim that the transfer of expertise can at best be partial because a large proportion of the research findings of the first world are military or industrial secrets. I'm quite sure that the late Dr Schumacher would have pointed out that most of this technology is quite inappropriate and indeed irrelevant to developing country needs.

The real problem is application

The real problem has nothing to do with shortage of research results, but the fact that they are not applied. In my own field of crop protection I get tired of pointing out that the application of presently available and published technology to small-farmer food-crop production in developing countries could quickly result in increased yields of at least 5–10%, and more in the long term—probably enough to eliminate malnutrition in large areas of the world. The use of first world research in developing countries almost always demands local research and development, but this essential requirement is often overlooked by both parties and is one of the greatest obstacles to the transfer of technology and expertise. A successful transfer must take into account not only the scientific and technical requirements but the socio-economic and cultural background as well where most of the difficulties arise.

I should not like it to be thought from the foregoing that I consider all is well with research cooperation arrangements between the first and third worlds, but it will not be improved by a one sided presentation of the issues. There is much scope for information gathering and if the Pugwash group can help develop that then it will be doing all of us a service. I say "all of us" deliberately: one of the main difficulties in this area is the paternalistic attitude of much of western science towards its developing country counterpart, and the feeling this engenders that we have nothing to learn from them. This is not only nonsense—it is unscientific. □

Pressure grows for US to resume chemical weapon production

David Dickson reports from Washington on a shift of emphasis in Defense Department thinking

PRESSURES are again growing in Washington for the United States to resume production of chemical weapons, on which a moratorium was imposed in 1969 following protests about the use of incapacitating and defoliant agents in Vietnam.

The pressures are the result of concern within the Department of Defense at the build-up of chemical weaponry by the USSR. They also reflect uncertainty about the progress of the chemical disarmament talks currently taking place between the US and the USSR in Geneva.

There has recently been a steady upgrading in the Defense Department's Chemical Corps, part of a general shift in focus towards possible non-nuclear conflict between the USSR and NATO forces in Europe. In addition, Defense Secretary Harold Brown announced last month that 900 Weteye bombs, the most up-to-date nerve-gas munitions in the US stockpile on which production was halted in 1969 and which had previously been scheduled for destruction, are to be retained because of their "deterrent advantage".

And sources in Washington now say that the administration is considering a likely request from the Defense Department for funds to initiate steps that could lead to the production of so-called binary weapons.

Binary weapons get their name from the fact that they contain two separated chemicals, each non-lethal on their own, which are placed in an artillery shell and combine on firing to form a nerve gas. (One design, for example, involves combining the liquid ethyl - 2 - diisopropylaminoethylmethylphosphonite with one of the solid dimethylpolysulphides.) R&D programmes have already been completed for one binary artillery shell, and are expected to be completed soon for another shell and a nerve gas bomb.

The Pentagon has been working on binary munitions since the late 1960s, intending that they should eventually replace the more conventional forms of nerve-gas delivery systems—for example since they are easier to handle, store and transport.

However budget requests for money to begin the production of binary munitions were denied by the US Congress in both 1974 and 1975. At the time Congress felt higher priority should be given to developing protec-

tive measures for US troops; it was claimed, for example, that reducing the effectiveness of a chemical attack would itself increase pressures for disarmament.

Many of Congress's demands for placing the emphasis on protection have now been met. Agreement to increase spending on protective measures against chemical attack was reached by the heads of NATO countries last month.

One reason given by Congress for rejecting the demand for binaries was that such a decision should depend on the progress of the bilateral talks on chemical disarmament which were originally agreed by President Nixon and Mr Brezhnev at their Moscow summit meeting in 1974.

These talks started in Geneva in August 1976, and have just recessed for the summer after seven months of discussion. Opinions about their progress vary. Some point to the agreement reached on a number of points of principle as indicating that important progress has been made. Others are more cautious. Professor Matthew Meselson, Cabot professor of natural sciences at Harvard University and editor of *Chemical weapons and chemical arms control* (published last

month by the Carnegie Endowment for International Peace, 11 Dupont Circle NW, Washington DC 20036) says that the talks have now reached a cross-roads, and that the real business is only just beginning.

A particular sticking point has been the problem of verification. Both sides now accept that this must be included in any agreement, and that it should be based on a combination of national and international arrangements. However, a communique issued last month admitted there was no agreement on verifying the destruction of stocks or a ban on manufacture.

Pressing for greater efforts towards chemical disarmament at the United Nations Disarmament Conference in New York two weeks ago, British Prime Minister James Callaghan repeated his promise that Britain, which has already tabled its own draft convention on chemical weapons, would accept inspection of relevant chemical manufacturing plants, as well as any other measures needed to underpin future agreements.

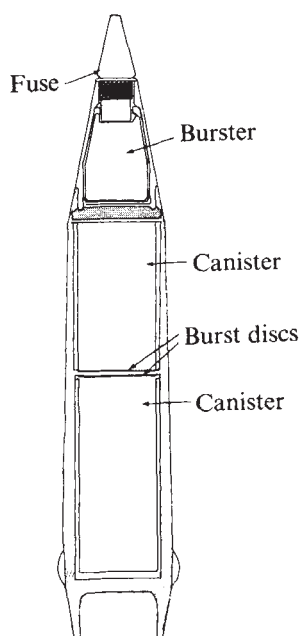
However, elsewhere such acceptance seems a long way off. With little chance of an immediate agreement, some Defense Department officials are using the opportunity to restate the case for upgrading the US's deterrent capacity. They are arguing, for example, both that the defensive measures requested three years ago by Congress have now been taken care of, and that many of the existing stocks of chemical weapons are deteriorating.

Two years ago a request to include \$15 million in the budget message to Congress to build a binary munitions production plant—although not to begin production itself, which would have required further Congressional approval—was rejected by President Ford largely on the grounds that it might prejudice the Geneva talks.

However budgetary recommendation for the fiscal year 1980 are now being prepared for submission to President Carter, and a similar request—although slightly larger to allow for inflation and other factors—is expected to be made again by the Defense Department.

Whether this request will have any greater success than its predecessor could depend on the case for cost-effectiveness of chemical weapons that the Pentagon, facing cost over-runs in more conventional areas of the Defense Procurement Programme, can make with the president's economic advisers.

But if it does get through in the president's submission to Congress, a rough ride can be predicted on Capitol Hill, particularly from those supporters of disarmament who claim that any such act can only increase the likely use of chemicals in war. □



Cross section of a binary artillery shell: will Carter accept a request that a factory be built capable of producing such weapons and other designs now being studied?

UK extends the law to genetic engineering

FROM 1 August, 1978 any scientist intending to carry out experiments in genetic manipulation in the UK will be obliged by law to notify both the Genetic Manipulation Advisory Group (GMAG) and the Health and Safety Executive (HSE). Notification will be made very much as it is under the recent voluntary system, which has been operated by GMAG for the past year and a half and which has resulted in almost complete cooperation. Application of the new regulations should therefore be painless.

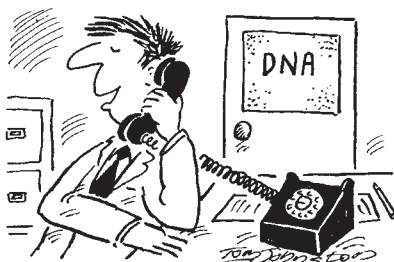
The new Genetic Manipulation Regulations have been made under the Health and Safety at Work Act. They arise directly from a recommendation of the Williams Working Party report of 1976 and were first drafted in August 1976. That draft, particularly in its provisional definition of genetic manipulation, was so criticised, if not ridiculed, that almost two years have been spent in wording the final regulations. Or, as John Dunster, deputy director general of the HSE, put it "Consultation is a very real process".

The final definition of genetic manipulation is said to have been widely approved and is in line with that accepted by the EEC and by several other countries. It reads: "the formation of new combinations of heritable material by the insertion of nucleic acid molecules produced by

whatever means outside the cell, into any virus, bacterial plasmid, or other vector system so as to allow their incorporation into a host organism in which they do not naturally occur but in which they are capable of continued propagation".

The other stumbling block experienced by those drafting the regulations was how to define 'work'. The problem is that not all students consider it to be a four letter word; indeed some carry out research work and yet are not employed and consequently have not come under the Health and Safety at Work Act. The solution in the Genetic Manipulation Regulations has been to extend the meaning of work to encompass those who are 'non-employed' such as students.

The compulsion encoded in the regulations begins and ends with the noti-



"I'm trying to cross a Health and Safety executive with a Genetic Manipulation adviser!"

cation of both GMAG and the HSE of intention to carry out genetic manipulation. It is, in theory at least, a voluntary matter whether or not any advice offered by GMAG (HSE will be relying on GMAG for that) is followed.

In practice, however, failure to comply with GMAG's suggestions is almost certain to lead to prosecution under the Health and Safety at Work Act. Information leading to such actions is expected to come either from the HSE's 'intelligence network', particularly safety officers in laboratories or via GMAG which includes two assessors from the HSE. The presence of these assessors has already been persuasive on rare occasions when there was reluctance to meet GMAG's advice.

The Genetic Manipulation Regulations will not be the last official word on the subject in the UK. Already a governmental Select Committee on Science and Technology is in the process of looking at genetic manipulation. Meanwhile, Leo Abse, a member of parliament, has criticised the new HSE regulations as being totally inadequate and providing no effective, enforceable control. Lastly, if the HSE considers that scientists are not complying with the suggestions of GMAG, it intends to incorporate the suggestions into an enforceable Approved Code under the Health and Safety at Work Act.

Peter Newmark

Soviets plan 'a new space event'

RECENT checks on the *Salyut-6* space station, at present operating automatically, showed all systems working normally. According to Moscow radio "a new space event" is expected shortly, which is presumed to include at least one non-Russian cosmonaut. The Polish and East German candidates for the flight have now completed their training; for psychological reasons, however, it has not yet been decided who will be scheduled to make the flight. The Hungarian team has now completed the first two months of training "successfully".

The manned *Interkosmos* programme, with the personal participation of citizens of the Soviet Union's Comecon partners has, naturally, been hailed as a political triumph. "The fraternal friendship and cooperation of the countries of the socialist community has gone beyond the limits of our planet and into the wide-open spaces of our universe", declared Mr Brezhnev.

Admittedly, there are some minor and unofficial doubts about the participation of the less influential member

states. All have been promised a flight for one cosmonaut within the current series of tests. No timetable has been announced, but if the programme is to be completed before the next solar maximum, it seems not improbable that the Mongolian and Cuban representatives, for example, will have to be content with a short space-ride rather than a record-breaking epic.

Commenting recently on the future development of space stations, Yuri Zaitsev, head of the Soviet Space Research Institute, noted that in the opinion of Konstantin Feokistov, himself a cosmonaut, the present generation of orbital stations have so many instruments aboard that it would be impracticable to build much bigger stations than *Salyut* at present.

On the other hand, Zaitsev added, "in future it may become more practicable to build stations which will work for decades with some 20-30 cosmonauts working in shifts. And as a long term objective, I could mention super-large, multi-purpose space complexes meant for a crew of 100 or more".

Zaitsev's remark is not the only hint

that the Soviet space planners now preparing more seriously for more manned missions beyond the earth's orbit. Results from the Czech team, who have been processing the results of algae studies aboard *Salyut-6*, note that no genetic changes attributable to weightlessness have so far been ascertained—a fact which "bodes well for man's extended flights in outer space".

And looking still further ahead to permanent lunar colonies, the latest issue of *Kosmicheskaya Biologiya i Aviakosmicheskaya Meditsina* reports that plants such as wheat, turnips, beetroot and radishes, grown in a quasi-lunar photocycle of 15 days light, 15 days dark gave a satisfactory yield of oxygen and food products, although the latter were somewhat less from than the "earth-cycle" control specimens. Since seeds from one generation of plants germinated to provide the next generation, the prospects for a self-sustaining colony (without the expensive imports of seeds characteristic of previous models) are felt to be extremely promising.

Vera Rich



Study begins on baby mammoth

WORK on the baby mammoth, discovered last summer in the Kolyma permafrost, in north eastern Siberia, is now under way. According to Professor N. Vereshchagin of the Historical Fauna Department of the Zoological Institute of the Soviet Academy of Sciences, there are two main lines of research.

The first is concerned with mammoth development. The Kolyma mammoth is the youngest so far discovered (7-8 months); previously, the youngest ever found was a 5-6 year old specimen discovered in Alaska. One team of investigators is therefore using the Kolyma find to study the "post-embryonic development (of mammoths), using X-ray photography to study dental development and the ossification of the pelvic bones and skull". To preserve the find for future studies of this type it is being mummified

by a paraffin-based substitution process.

The second main series of tests is designed to study protein evolution. Investigation of the mammoth proteins, it is hoped, will throw light on how protein structure has developed. Another interesting problem, according to Professor Vereshchagin, is how animal protein endures under conditions of permafrost.

Although Vereshchagin stresses the "unique" and "remarkable" nature of the find, which came to light last summer under the scoop of a bulldozer, and was saved only by the astuteness of the driver, he also points out that the Kolyma-Berezovka permafrost area is "as it were a natural reserve of ancient fauna". Other bulldozer drivers, it is implied, should keep a sharp look out.

Vera Rich

Scientist to head Peking University

CHOU Pei-yuan, a distinguished Chinese physicist, has been appointed president of Peking University. The appointment is significant, because Chou was a prominent critic of Mao's educational policy. Even while Mao was in power, he argued strongly that science should be given a prominent role in university life and clearly distinguished from practical subjects like engineering. He suffered "criticism" for his ideas.

Chou, a general relativist and theoretical physicist, studied at the University of Chicago and elsewhere in the US in the 1930s. The Nobel prize-winner, Yang Chen-ning, was his student at Tsing-Hua University (then

People's Republic of China



Chou the teacher: significant appointment

National South-West Associated) from 1938-42. Chou returned to the US for a few years after the end of the Second World War but spent his career after the revolution in China. He joined the Communist Party in 1959.

Bangladesh scientists strike for more pay

SCIENTISTS and technologists in Bangladesh are disappointed with the status and pay scales awarded them by the National Pay Commission at the end of last year. They feel that the pay commission has failed miserably to evaluate their importance in the development of the nation and that it has given undue importance and unjustifiable emoluments to civil servants.

For the first half of May, scientists went on strike, stopping work for two hours every morning. In the middle of the month, however, strike action was withdrawn when the Implementation Division of the Government began considering the scientists' demands for more pay.

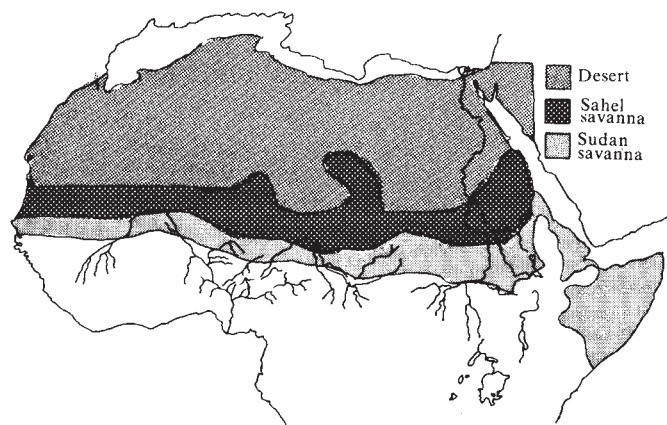
In response to the Pay Commission, a group of Bangladesh scientists have formulated a new model of 'service structure' for scientists, engineers and medical and agricultural graduates working in government autonomous and semi-autonomous organisations.

A really capable scientist, says the group, even though he may be working in a laboratory, should be able to hold the highest position and status in public service without necessarily holding a high administrative position. Scientific administrators, who are scientists themselves, should also have their own pay scales, the scientists say, and act as public contacts. The rigidity of the structure of posts should not hinder the upgrading of a capable scientist: posts should be fitted to the person rather than the person to the post. The model proposed by the Bangladeshi scientists thus differs radically from the existing pyramid structure of the civil service.

M. Kabir

Professor Yang, who is now at the State University of New York at Stony Brook, has visited Chou many times in recent years but was surprised at his appointment. "There have been very few scientists appointed president of Chinese universities" Yang said. Chou was "an excellent choice. He is a greatly respected educator and scientist in China. His appointment underlines China's determination to forge ahead in science and education".

Chou, in an article in *Kuangming Daily* (6 October 1972), argued that it was not the physicists' job to produce direct solutions to practical problems, though there could be "spin-off"—like atomic energy. His new appointment seems to represent an endorsement by Chairman Hua's government of his views. □



What next in the Sahel?

THREE years after the great drought that brought the Sahel into the headlines opinion as to the future of the two million square kilometre region are still sharply divided. This was apparent at a recent meeting held in London under the joint auspices of the Royal Geographical Society and the Royal Society of Arts, curiously entitled 'Sahel ecology: possible improvement'.

The meeting brought together a heterogeneous collection of speakers—botanists, geographers, zoologists, but apparently few ecologists—from several countries, although only one was from the region itself. After more than eight hours of papers and discussion, little new was suggested that could be expected to improve conditions in what, Professor A. T. Grove pointed out, is one of the poorest inhabited regions of the world.

He suggested that the Sahel might never again be as productive as it had become by the mid-1960s. By that time, 30 years of uncontrolled 'development', largely in the form of well-drilling and veterinary activities, had led to a livestock population of about 10 million cattle, 10 million sheep and uncounted goats. With a human population given as 10 million, but perhaps as much as 25 million, the carrying capacity of the region had been reached, if not exceeded.

Even so, the drought of the early 1970s cost less in human suffering than the last such major event in 1913, although livestock losses were very much greater. (It is perhaps worth noting that the Food and Agriculture Organisation had been warned, by one of its own staff members, of the possibility of some such disaster as a result of its policy of encouraging increased livestock production in the Sahelian countries. The warnings did not suit the organisation's livestock experts however, and went unheeded).

Some speakers maintained that the drought was not so much due to a shortage of water as to lack of food for both livestock and humans. As people and their livestock were driven from the northern Sahel by lack of sustenance, they caused grazing and other pressures on the more productive south resulting in rapid degradation and erosion of soils already exploited to the maximum. It is in the south that the most serious damage may have been done.

What is needed to rehabilitate the region is the business of the Sahel Research Institute at Bamako, Mali, whose director, Dr Ousmane Silla from Senegal, was the region's sole contributor to the meeting. The institute, now getting under way with help from various United Nations and other agencies, is seen as the coordinating centre for research in the individual Sahelian countries, where funds and expertise are too scarce to be wasted on ill-conceived projects or duplication of effort.

The institute is to give priority to 'harmonisation' of research and to training indigenous young scientists in the disciplines applicable to research in the region, where most experience and knowledge at present comes from foreign experts. An early start is planned on projects which include studies of the water balance in the region; the crops most suitable for the various parts of the region; improvement and 'commercialisation' of livestock; and the human, ethnic, and social problems of the region as a whole, including those of health and nutrition. The development of freshwater fisheries was also mentioned—a field in which FAO is interested in the Sahel—but Dr Silla had no comment to offer on the suggestion that what the Sahel's agriculture needed for more effective cropping was mineral nutrients rather than water.

Apart from Dr Silla's presentation, appreciation of the need to consider the human population was more apparent in the discussions than in the papers themselves. Papers from three visitors from the Office of Land Studies of the University of Arizona suggested that the Sahel's problems could be at least partly solved by the introduction of large-scale industrial crops such as the oil-bearing jojoba (*Simmondsia sinensis*) and rubber-producing guayule (*parthenium argentatum*). Among those disagreeing with this approach was Professor Cloudsley Thompson, who pointed out that all such big schemes in this type of region had failed, even that in the Gezira being by no means as successful as had been claimed. His suggestion would be for many small, diverse projects using local resources to fulfil local needs. The failure of any one would not be disastrous. Dry farming should be developed, particularly in view of the risks of parasitic diseases attendant upon large irrigation schemes, and of the availability of modern techniques for dune fixation and desalination.

No one expects a miracle in this difficult and remote region. Perhaps the biggest danger lies in its exploitation by outside interests. Climatologists at the meeting seemed to feel that the danger of another series of drought years was not imminent, but maximisation of production in normal times will always lay the Sahel open to disasters such as that which has inspired so much talk (and possibly, so little action) since 1975. Given disinterested advice and aid the peoples of the Sahel are perhaps better able to decide what is best for their own future than are the innumerable agencies, international, bi-lateral and charitable, at present offering assistance and not always very practical suggestions.

Peter Collins

Wave power boosted in UK energy hand out

LAST week the UK Department of Energy (DoE) produced its long-awaited reply to two reports of the House of Commons Select Committee on Science and Technology on alternative energy—and the Severn Barrage. In the guise of a White Paper called 'The development of alternative sources of energy', the reply announces the allocation of £4.5 million for R&D on wave, wind and geothermal energy and the provisional allocation of £1.5 million for further studies to assess tidal barrage schemes in the Severn estuary. A Severn Barrage committee under the chairmanship of Sir Hermann Bondi, Chief Scientist in the department, is to be set up to advise the DoE on which studies it should spend its money.

The White Paper's recommendations are broadly in line with those set out in the two select committee reports. They acknowledge that alternative energy sources should be developed with "urgency and determination". The White Paper goes on to say that because these sources are at an early stage of their development, "the limitation to making faster progress is not the level of funding but the state of the technology involved". According to Sir Hermann Bondi however, alternative energy sources will become "hungrier and hungrier" as their development progresses and the DoE is "to keep the level of funding under close review and be prepared, in the light of progress, to make further sums

available for the more promising lines of development".

The lion's share of the £4.5 million is to be spent on wave power, the alternative energy source with the greatest potential for large scale electrical generation in the UK. £2.9 million is to be added to the £2.5 million programme due for completion in October. Half of it will go to support device development, £300,000 will be used to support collaborative work with other countries (especially Japan) through the International Energy Agency and the remainder will be used to tackle the environmental and engineering problems applicable to all types of device.

Wave power is a "high risk, high reward" energy source according to Sir Hermann Bondi. If developed successfully, it could harness the energy in the particularly heavy seas around Britain with the advantage of a supply matched to the demand (the heaviest seas, and hence the most energy, occur during the winter). However, the engineering problems are formidable and the economics of a technically feasible device would be difficult to work out. Nevertheless tenth-scale models of two devices have already been built and the DoE White Paper says that subject to satisfactory progress expenditure will increase over the next three or four years and a single device will be identified for concentrated development. The programme is to be reviewed each spring.

Research on wind power comes in for an additional £806,000. £341,000 is to be spent during the next year on design and component testing of a 60 m diameter, 3.7 MW commercial-size prototype 'aerogenerator' (the new word for windmills). If this stage is successful, the DoE will start a three year programme costing about £2 million to build and test a prototype. The remaining £465,000 will be spent over the next two years on development of a vertical axis aerogenerator and wind energy from lowland and off-shore sites.

Although geothermal energy is not likely to be providing energy in the UK until after other alternative sources have been developed, the DoE has allocated £856,000 for further geological, geophysical and hydrogeological surveys and studies of heat flow. The solar energy programme, however, does not come in for any of the hand out. It was increased to £6 million last year when an extra £3.6 million was made available and the DoE feels that no other measures are needed at present to further stimulate industry with its development work. However the White Paper does not go along with the select committee's recommendation that funds should be available to encourage installation of solar water heaters. The current state of the technology does not support this case, it says.

Judy Redfearn

Bacterium makes insulin

THE synthesis of mammalian insulin by a bacterial cell, one of the most canvassed examples of the benefits of gene transplantation techniques, is at last a reality. In a lecture at the University of Chicago last week Professor Walter Gilbert of Harvard University reported the construction in his laboratory of bacteria producing small quantities of rat proinsulin, the immediate precursor to biologically active insulin.

The genetic information for the rat proinsulin was inserted into a bacterial plasmid which encodes the bacterial enzyme penicillinase. The completed nucleotide sequence of this plasmid is known, and the modified plasmid was resequenced after insertion of the proinsulin DNA to check that the inserted fragment was in the correct orientation and reading frame to be translated as part of the penicillinase protein.

Penicillinase was chosen as the 'carrier' for the foreign protein as it is one of the proteins naturally secreted from the bacterial cell. When the

modified plasmid was introduced into a suitable strain of *Escherichia coli* the bacterium synthesised minute amounts, around 100 molecules per cell, of the modified protein from which the proinsulin could be recovered.

The idea of inducing bacteria to synthesise foreign proteins by hiding them in a natural bacterial protein was first used successfully to synthesise the brain hormone somatostatin last year. In that case the somatostatin coding sequences were chemically synthesised; in this case the proinsulin DNA was apparently copied from the abundant insulin messenger RNA produced by an insulin-producing rat tumour. □

MPs seek to see Shcharanskii

ANATOLII Shcharanskii (right), the Moscow cyberneticist and human rights activist, was arrested in March 1977 for alleged subversive activities and has since been held incommunicado in Lefortovo prison.

The International Committee for the defence of Anatolii Shcharanskii, which includes members of both houses of Parliament and representatives of the arts and professions, last Thursday issued a statement calling for his release. The committee is to seek visas for its co-chairmen MPs John Gorst (Con), Helene Hayman (Lab), Russell Johnston (Lib) to visit Shcharanskii. In the event of a trial they will seek to send a delegation from both houses of Parliament to observe the proceedings in accordance with international precedent. □



correspondence

Developing countries need appropriate science policy

SIR,—The eight problems regarding the relationship between researchers in the first and third worlds, summarised in Robert Walgate's article (2 March, pages 8–9), constitute very important challenges to the developing countries themselves. Contrary to the popular belief that research is an expensive ivory-tower activity, it is developing countries that must spend relatively more of their resources on research in order to accelerate their development.

Developing countries must establish and finance scientific policy bodies made up of local scientists, who are in the best position to interpret real development problems into the necessary scientific and technological requirements. Local scientists must not only carry out basic research, they must also act as focal points for the processes of transferring scientific knowledge towards solving development needs. They must not only assist their nations in defining development problems, but they must also be sympathetic to and guided by those development needs in organising their research activities.

One of the biggest drawbacks in developing countries is the lack of continuous and effective transfer of scientific knowledge to all people, especially to such key people as teachers who can be very effective in reaching the general public. There is a tendency for knowledge to remain a possession of the more educated, urban and industrial/commercial communities. Local scientists have a vital role to play in effecting this transfer of knowledge so that the creative potentials of people, including rural communities, can be enriched and facilitated for development.

It is in achieving all this that the assistance of the developed countries needs to be sincerely given. All research done in a developing country should make a definite contribution to the development of that country. This can only happen if the developing countries are assisted to have effective science policy bodies and scientists whose operational capabilities are enhanced and maintained through provision of adequate technical staffing and financial resources.

Yours faithfully,

JOSEPH MAINA MUNGAI

National Council for Science
and Technology,
Nairobi, Kenya

Dangers of 'dissident' correspondence

SIR,—As a practising physicist with some personal contact with Russian scientists, I share the shock and horror which must be felt by everyone over the cruel severity of Orlov's sentence, and indeed his apparent lack of crime (in western terms) in the first place.

However, I fear that your

encouragement (25 May, page 255) to western scientists to express their disgust to Soviet colleagues through the several channels of communication available to us may be seriously irresponsible in one regard. I am concerned that to write to a Russian colleague on the subject of human rights and political dissension in the Soviet Union may invite upon that colleague official disapproval from the Soviet authorities. We cannot be sure of the extent of surveillance and censorship of incoming letters to Soviet scientists, but we can be sure that the Soviet bureaucracy would take a dim view, if they did find out, of one of their scientists being involved in 'dissident' correspondence with westerners. At the least we may be embroiling a friend or colleague in a political issue in which he or she does not wish to become involved, perhaps for very good personal or family reasons. Moreover, if the scientist to whom we write has travelled in the West previously the suspicion may arise that during his visits he has indulged in anti-Soviet discussions, and we may well be putting him on the black list as far as further travel to the West is concerned. And quite possibly in the extreme case we might be seriously putting at risk the safety or security of our colleagues or their families.

We cannot be sure, of course, that any of these things may happen. But although the treatment of dissidents like Orlov is atrocious, do we have any right to interfere and intrude in a self-righteous way into the personal lives of Russian scientific colleagues? There is an element of a blind 'crusading' or 'God on our side' attitude in doing this if we do not carefully investigate the ramifications and implications fully of such action.

For these reasons, and that of pure effectiveness, I believe that the best course of action is for those of us who are concerned about the issues raised by the Orlov case and others to encourage our official agencies, such as the Royal Society, the Institute of Physics and Anglo-Soviet organisations, to contact their counterparts in the USSR and the Soviet government about these travesties of human rights. In this connection I was encouraged to learn in an accompanying article that the Royal Society has already spoken out on the Orlov sentence.

Yours faithfully,

JOHN E. HARRIES

Surbiton, Surrey, UK

Parkinson's Law in the Antarctic

SIR,—While I agree that there is a general tendency for the number of administrators in a scientific organisation to grow, I believe that in developing a thesis it is incumbent upon the originator to use facts which he not only believes to be correct, but to ensure that those facts, when they are used for comparative

purposes, are comparable in origin. This does not appear to be so in the case of the data used by Dr Moss in his letter (18 May, page 184). I can only speak with knowledge of one of the fourteen NERC component bodies, the British Antarctic Survey, for which he gives as total staff 337—scientific staff 170—addresses one.

In examining these figures we need to determine the difference between administrators and non-scientific staff who are not administrators. Dr Moss appears to confuse the two. Ships' crews, aircrew, mechanics, radio operators, electricians, cooks and carpenters are certainly not administrators. The BAS staff in these categories number 127. But since none of the scientific work in the Antarctic could be done without them, it is difficult to see how the statement "the number of administrators within a public organisation tends to increase irrespective of the amount of external work done by that organisation" can be said to apply.

If it is thought that it does, then their counterparts in an organisation working entirely in the UK, should also be included—wives, electricians, transport staff, mechanics, switchboard operators, cleaners, and indeed many others who in this country provide the services on which scientists depend. This would make a nonsense of his thesis. I therefore suggest that, in one case at least, his concept is based on comparing unlike with unlike.

Another factor with which much play is made is the number of 'addresses'; BAS being said to have one. In fact the survey has its headquarters in Cambridge, an office in the Falkland Islands and five Antarctic stations. It also runs two ships and two aircraft. The operation of all these, including their requirements for food, buildings and equipment in the field, are provided for by a total administrative staff of only 40.

I would say to Dr Moss, as regards the British Antarctic Survey,
'O most lame and impotent conclusion.'

(*Othello*, Act ii, sc. 1.)

Yours faithfully,

V. E. FUCHS

Cambridge, UK

God's bonds

SIR,—Why do nucleic acids have 3'5' phosphodiester bonds? This question was resolved by Dhingra and Sarma in a recent issue of *Nature* (272, 798 (1978)).

Earlier, the irreplaceable late Gordon Tomkins quipped (as quoted by Paul Sigler):

"Twos and fives are made by fools
like you and me,
and only God can make a five
and three".

Yours faithfully,

HENRYK EISENBERG

Rehovot, Israel

news and views

Giant problems

from M. G. Edmunds

COOL giant stars are very complex physical systems. From the observational point of view, the spectra of their atmospheres are hard to interpret, and theoretical predictions of their internal evolution is made difficult by the inadequacies of mathematical models of the convective processes occurring inside them. Although the final supernova explosions of massive stars are believed to be the main chemical element factories in the Galaxy, nuclear synthesis during the far more peaceful giant stages of stellar evolution contributes quite a large fraction of the isotopes of the elements in the periodic table. Although recent research has not resulted in a full understanding of these cool stars, it has at least highlighted the areas of uncertainty and suggested the direction of future work. Particularly interesting have been calculations of element synthesis by Icko Iben and James Truran (*Astrophys. J.* **220**, 980; 1978), together with the interpretation of observational data on the intrinsic brightness and spectra of these stars by John Scalo (*Astrophys. J.* **206**, 474; 1976; Scalo & Ross *Astron. Astrophys.* **48**, 219; 1976).

On the basis of the features which are prominent in their spectra, cool giants can be classified into three major types. Most stars are 'M' stars, showing strong absorption bands due to metallic oxides, particularly TiO. Less frequent are carbon (or 'C') stars which have bands of carbon-based molecules, for example CN and C₂. Finally, a small fraction of cool giants are classed as 'S' stars, which again show mainly metallic oxides, but in this case the metals involved (for example, ZrO, YO) have a much lower abundance in the interstellar medium (and hence in the material out of which the stars formed) than either the titanium or carbon which dominate the spectra of M and C stars. The physical

differences between these stars have long been thought to be due to chemical composition, as examples can be found in which the other variables influencing the spectrum of the stellar atmosphere—the temperatures and gas pressures—seem to have similar values for all three types of star.

The critical parameter is undoubtedly the carbon to oxygen abundance ratio. If oxygen dominates over carbon, then molecular equilibria calculations indicate the dominance of molecules seen in M stars; if carbon dominates over oxygen, then nearly all the oxygen becomes tied up in CO, and carbon molecules dominate as in C stars. The interesting consequence of Scalo and Ross's calculations, which combine band strength estimates with molecular equilibria, is that it may be possible to explain the dominance of oxides like ZrO and YO in the spectra of S stars, simply by demanding that the carbon abundance be very nearly equal to the oxygen abundance, and without demanding that the atomic abundances of Zr and Y should be enhanced in S stars. These oxides just become favourable due to their comparative stability when titanium is having to fight hard for the oxygen against competition from carbon. This is not to say that Zr and Y abundances are normal in S stars, indeed, analysis of their atomic line strengths would suggest that they may be enhanced by up to a factor of 20, but it does remove the constraint that S stars necessarily have to have large enhancements of these elements in order to display their characteristic spectra.

Carbon stars, as well as S stars, show evidence of abundance enhancements of elements in addition to carbon. The elements involved have nuclei with particularly low cross sections for reactions with neutrons, and their abundance is believed to build up during the reaction chain resulting from irradiation of iron group nuclei with neutrons. The flux of neutrons has to be sufficiently small (that is,

'slow' neutron capture) to allow each neutron capture product nucleus to β -decay, if they are unstable on time-scales of the order of 10^4 yr, before the addition of another neutron. The products of this chain are known as 's-process' elements—a somewhat confusing title in this context, since the nomenclature of 'S' stars is purely arbitrary, and was made years before the s-process was thought up. A major problem has been to find out what chain of nuclear reactions during the cool giant stage of stellar evolution is capable of giving the neutron flux required for the s-process, and investigating whether the stars would be capable of mixing the reaction products up into their atmospheres where they could influence the spectrum.

During its evolution as a cool red giant, a star can reach a stage where its energy sources are in two separate shells around a quiescent core. The outer of the two shells is processing hydrogen into helium; the inner shell processes helium into carbon. This structure can become unstable, and a phenomenon known as 'thermal pulsing' or 'helium shell flashing' occurs in which the temperature of the helium-burning shell periodically rises, with intervals of perhaps a few thousand years between the thermal pulses. Iben and Truran have been investigating the synthesis of s-process elements during these pulses, on the assumption that the source of neutrons is a series of reactions starting with ¹⁴N (left over from previous nuclear processing in the star) and involving capture of α -particles. It is the last stage in this chain—the conversion of ²²Ne to ²⁵Mg—that is accompanied by neutron emission. Their conclusion is that the elements can be synthesised and mixed to the surface, but only in stars which have sufficient mass to exceed a particular intrinsic brightness. The calculations are admittedly somewhat uncertain because the theory of convection is at present so primitive—and it is these convective motions which

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must dredge up the newly-made material to the surface. But the argument against the relevance of this particular neutron source in stars of lower brightness also rests on the temperatures which have to be attained during the thermal pulses if element synthesis is to be significant.

Although much of the s-process material in the Galaxy is probably produced in this way, it does not explain the observational evidence (assembled by Scalo) of carbon and S stars of considerably lower brightness than the minimum required to give conditions in which the ^{22}Ne mechanism will work. This mechanism also predicts a steady correlation of enhancement of carbon and s-process elements. This might be all right for carbon stars, but not for S stars, where the s-process elements are sometimes far more enhanced than the carbon. As indicated above, too much of an increase in the carbon to oxygen ratio would change an 'S' spectrum into a 'C' spectrum. Iben and Truran therefore suggest a second mechanism to explain the lower luminosity carbon and S stars. When low mass stars begin their red giant evolution, the helium core left over from nuclear reaction of hydrogen during their life as main sequence dwarfs can ignite rather violently. This sudden switching-on is known as 'the helium flash', but it is not to be confused with the helium shell flashing of later evolution. If the flash occurred in some stars in such a way that a little unprocessed hydrogen were mixed into the core, a reaction chain starting with proton capture by any ^{12}C in the core would give rise to

neutron irradiation without greatly increasing the carbon abundance. The s-process elements could be mixed up to the surface during later evolution, even if the star were not as bright as required for the ^{22}Ne chain to function.

This second mechanism may well explain the 'barium' stars, which are rather hotter giants than those considered here, and which also show excess s-process elements, including barium. But problems remain in trying to account for S stars as cooler more evolved barium stars by this mechanism. The evolution of a star from its helium flash to the stage where it should look like a cool giant S star would take longer than the time required for the unstable s-process isotope ^{98}Tc of technetium to decay. Yet this technetium is observed in the atmospheres of some S stars, and must therefore have been produced and mixed up more recently than the helium flash.

Undoubtedly, much work remains to be done. Improvements in convection theory are badly needed. More reliable abundance analyses of cool giants are required to sort out just which elements are enhanced. Such analyses are difficult, as these are large, tenuous atmospheres with hideously complex opacity sources and it may be necessary to dispense with most of the assumptions of normal stellar atmosphere models—plane parallel geometry, local thermodynamic equilibrium, horizontal homogeneity. Even hydrostatic equilibrium may have to go, as many of these stars are variable and may have shock waves propagating through their atmospheres. □

As the nucleotide sequence of the RNA was known, they knew that all four of the valine codons were used in the specification of this product. Thus, the wobble rules suggest that at least two different valyl-tRNA^{Val} species with two different anticodons should be required. They found instead that the protein was synthesised and all four codons read when only one such purified species was added. Controls were run and various clever dodges used to eliminate possible snags in the interpretation, so it is hard to avoid the conclusion that the tRNA species that they used were recognising the first two bases of the codon (GU) but ignoring the third. This mode or response they have dubbed "two out of three" reading. Bergquist, they note, made similar observations a decade ago on the glycine family of codons using R17 RNA, but this case the conclusion was more tenuous in that the sequence of the messenger was at the time unknown.

Presumably "two out of three" reading cannot occur with codons that do not belong to families, for if it did translational errors would be rampant. This suggests that there is something special which distinguishes family codons from non-family codons. The question is what? Lagerkvist gropes towards the answer to this in a second paper (*Proc. natn. Acad. Sci. U.S.A.* **75**, 1759; 1978). Here he supposes that "two out of three" reading is only likely if the interaction between codon and anticodon in the first two positions is sufficiently strong irrespective of any possible pairing in the third position. He therefore decides that if only A.U pairs are possible in the first two positions, "two out of three" reading should not take place and such codons could safely be used as non-family codons. Inspection of the code shows this to be the case; indeed inspection also shows that if the first two pairs are both G.C the sixteen codons involved all belong to families. Nevertheless, the plot is deeper than this, for there are four families that only have one G.C pair in the first two positions (and thus 'mixed' codon interactions as he terms them), whereas the remaining four sets of mixed codons are used in non-family codons. Lagerkvist only notes that in the mixed codons used by families, the middle base turns out to be pyrimidine, but the significance of this is not clear.

The first part of Lagerkvist's explanation requires that G.C pairs in codon-anticodon interactions lead to an enhanced stability, as in double-helical DNA. This presupposition itself is unfortunately cast into some doubt as a result of the work of Grosjean *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **75**, 610; 1978). As the next best thing

Tilting at wobble

from E. G. Richards

THE decipherment of the genetic code is in all the textbooks; so is wobble hypothesis which describes how the codons in the RNA are recognised by tRNA molecules. All seemed well until recently, when Lagerkvist and his colleagues at Gothenburg conjured up what looks like a serious anomaly (Mitra *et al.* *J. biol. Chem.* **252**, 471; 1977). The wobble hypothesis requires that the first two base pairs between the codon of the RNA and the anticodon of the tRNA are ordinary A.U or G.C pairs but certain additional pairs are allowed in the third wobble position. Thus, according to the rules, U.G pairs are allowed in the third position as well as pairs between I in

the anticodon and A,C or U in the codon. Lagerkvist introduces first the notion of a family of codons; this is a set of four codons differing only in the third position and all coding for the same amino acid. The valine codons constitute one such family but there are seven others. The rules imply, if strictly obeyed, that at least two anticodons—and thus at least two tRNA species—are required to recognise all four members of a family. What Mitra *et al.* seem to have shown is that one species may be sufficient.

They set up a cell-free protein-synthesising system derived from *Escherichia coli* which was dependent on added valyl-tRNA^{Val}. They used as messenger MS 2 RNA, which in the conditions of their experiment led to the synthesis mainly of coat protein.

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to studying interactions between tRNA molecules and codons bound to the ribosome, they investigated the binding constants for the interaction of a large number of pairs of tRNA species which have complementary anticodons. As the rates of formation of such complexes are all roughly similar, the binding constants are reflected in their rates of dissociation and thus in their mean lifetimes. These they have measured by temperature-jump relaxation techniques. By and large, the results show that interactions involving base pairs allowed by the rules have lifetimes in excess of 10 ms, whereas those involving 'forbidden' pairs are more ephemeral than this. One surprise is that the stability of a complex does not increase regularly according to the number of G.C pairs involved, and it is supposed that there must be some effect that increases the stability of A.U pairs. Stacking effects contingent on the sequence of bases may be important, as may the nature of the bases next to the anticodons.

Unfortunately, it is not possible to examine in detail the suggestions made by Lagerkvist to rationalise "two out of three" reading, as of necessity in the complexes studied by Grosjean *et al.*, two "third-position interactions" are involved in each complex. Whether Lagerkvist is on the right lines or not will only be decided by the study of stability constants using a more realistic system. □

Lunar atmosphere past and present

from David W. Hughes

GEOLOGICAL evolution has played a major role in the fashioning of the lunar surface, and the phases are fairly well known now. First, about 4.7 aeons ago (an aeon is 10^9 yr) the Moon was formed near the Earth, probably by gravitational accretion of planetismals or possibly by fission from the infant Earth. Tidal and accretion energy caused the outer regions of the Moon to heat up to temperatures over 1000°C . This caused differentiation and produced a global crust which can now be seen as the lunar highlands. During this period 4.6–3.7 aeons ago the random influx of large planetismals produced the circular mare basins. Between 3.7 and 3.2 aeons past a secondary differentiation occurred, caused by repeated localised partial melting of the outer 500 km of the crust. This produced a basaltic magma

which erupted and flowed repeatedly to form the maria. From 3.2 aeons ago to the present time we have a Moon which is cold and rigid to a depth of about 1000 km. Below this region the pressure and temperature becomes sufficient for magma generation. The surface regions are troubled by occasional minor volcanism, this being exhibited as gaseous emissions (known as lunar transient phenomena) around certain craters and as accompanying seismic activity. Mild moonquakes are periodically triggered by the Earth's attraction when the Moon is at the perigee of its orbit. Meteoroids from the dust cloud around the Sun and meteorites, probably from asteroidal fragmentation, impact sporadically with the surface producing craters. This continual cratering produces a soil-like regolith which gets turned over by subsequent impacts, a process known as lunar gardening.

Where does the lunar atmosphere come into this picture? Most researchers believe that the Earth and Moon were born from 'common stock', the fundamental difference being that the Earth is 81 times more massive. The first atmosphere on Earth was produced by the outgassing of volatiles and carbonaceous compounds during the times of igneous activity. The first products were probably H_2 , CH_4 and NH_3 . Gradual oxidation changed these to H_2O , CO , CO_2 , N_2O and NO_2 . Life and photosynthesis produced our present-day N_2 and O_2 atmosphere. It is highly probable that similar gaseous emission would have occurred on the Moon during the periods of intense volcanic activity 3.7–3.2 aeons ago. We are left with two problems: where did the atmosphere go and how long did it take to leave?

The first question is answered easily: the atmosphere escaped into space. Planets with similar densities have escape velocities proportional to the one-third power of their mass. The Moon's escape velocity is thus a quarter that of Earth's, its gravitational energy being insufficient to overcome the thermal kinetic energy of the emerging atmospheric molecules. The present minor lunar atmosphere has a concentration of about 2×10^5 molecules cm^{-3} (about 10^{14} less than Earth) at night, decreasing by a factor of about 20 during the lunar day. The solar wind is thought to be the main source.

The second problem still awaits a solution. Traces of atmospherically induced phenomena in the present-day lunar surface features are hard to find. It is even more difficult to obtain any idea as to the time scale and density

of this atmosphere.

Yu B. Chernyak tackles this second problem in a rather interesting way on page 497 of this week's *Nature*. He considers the effect that a lunar atmosphere would have on the impact velocities of micrometeoroids and their ability to produce craters in lunar rocks and glassy spherules.

The effect of the Earth's atmosphere depends drastically on the mass of the incident meteoroids. When a particle of mass less than 10^{-6} g enters the atmosphere, the heat absorbed by the particle from atmospheric friction is insufficient to raise the particle to boiling point. It is retarded to the free-fall velocity and drifts to the surface. More massive particles do reach boiling point and ablate, the mass loss producing a meteor train; the greater the mass loss rate the more intense the train luminosity. The particle is decelerated during train formation, and for particles with incident masses greater than about 10^7 g some tangible mass remains after it has been decelerated to free-fall velocity. This hits the ground as a meteorite, the Hoba meteorite being a perfect example. This 60-tonne iron meteorite was found near Grootfontein, South-West Africa, resting on the ground having only made a slight depression. In contrast, the meteorite responsible for the Arizona meteorite crater was so massive (250,000 tonnes) that ablation and atmospheric retardation made an insignificant change in its cratering ability. A crater 128 m across and 180 m deep resulted.

The Moon's atmosphere would produce a related effect. Chernyak first points out that the differential slope of the mass distribution of the lunar meteoroid influx, obtained from crater size distributions on some lunar glassy spherules, decreases to zero in the mass range 10^{-7} to 10^{-11} g. This is interpreted as indicating that no particles of this size range hit the lunar surface at their 'in space' orbital velocities for about 10^5 yr, the exposure time of the samples. It is highly unlikely that the mass distribution of the cosmic dust cloud has changed significantly in the past 10^5 yr, so the deficiency in this size range is probably caused by atmospheric retardation. Chernyak calculates that the atmospheric column density required to retard particles in the 10^{-11} to 10^{-7} g size range, but not the larger ones, is in the region of 0.3×10^{-2} to 0.7×10^{-2} g cm^{-2} . Thus, the atmospheric mass in a column of unit area cross section on the Moon would have been about 2×10^6 times less than the present-day Earth atmosphere value. The samples under investigation were cratered when the Moon had an atmosphere, became buried in the regolith and thus shielded from the

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normal $5 \times 10^{-8} \text{ cm yr}^{-1}$ erosion that exposed particles suffer when there is no atmosphere.

Chernyak also considers the granularity of the lunar regolith. Compared with the mass distribution of the micrometeoroid influx the regolith is found to have a depleted population of small particles and what is more this depletion is enhanced as a function of depth (crudely, this means as one goes back in time). The present influx has too many small particles to produce the observed regolith granularity. Calculations indicate that an atmosphere of column density $10^{-2} \text{ g cm}^{-2}$ would produce the observed cut off, a value refreshingly close to the one obtained from the crater count analysis.

So according to Chernyak, the Moon had an atmosphere sometime in the past 10^8 yr with a total mass 5×10^7 that of the present lunar atmosphere. We are still left with the problems of finding out when the atmosphere occurred and for how long it lasted. It will be also of interest to consider whether this atmosphere could affect lunar geological, geographical and regolith properties other than microcrater densities and soil particle size distributions. $\square$

Wet semiconductor devices

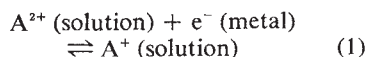
from Laurence Peter

WHY do many undergraduates find thermodynamics more difficult to grasp than other topics in physics and chemistry? Perhaps the abstract character and formal elegance of the thermodynamic approach lack appeal, but whatever the reason, it is refreshing to see that thermodynamics is alive and well and providing valuable insights into a range of new problems which are far removed from the rather dusty steam engines of Sadi Carnot.

Semiconductors are commonly thought of in the context of silicon electronics, but of course many materials are semiconducting, and potential fields of application extend, for instance, to solar energy conversion. Solid state devices have traditionally exploited the characteristics of contacts between semiconductors of different electronic properties (n and p-type materials) or between a semiconductor and a metal (Schottky diodes, MIS devices). Recently, however, there has been renewed interest in semiconductor-liquid junctions, and undoubtedly the next few years will witness rapid progress in this field. The

chemically sensitive semiconductor devices (CSSDs) analysed by Bergveld *et al.* (*Nature* **273**, 438; 1978) are good examples of the flexibility and potential utility of liquid junction devices for chemical analysis, and the spate of recent publications on liquid-junction solar cells indicates that we are likely to hear more about 'wet' semiconductor devices in future.

What happens when we bring a solid and a liquid into contact? The charged species in the solution are ions and therefore electrons can only cross the interface if some reaction occurs. By contrast, when two solids are joined, equilibrium is established by electrons which can flow across the interface and set up a potential difference to compensate for the initial difference in the free energies of electrons on each side. The free energy of an electron in a solid phase is described in terms of the Fermi energy level E_F (we have to be careful to include kinetic and potential contributions to E_F). On the other hand, the concept of the Fermi level is not often used in association with electrolyte solutions. The description of contact phenomena is very simple in terms of Fermi levels, and it is worthwhile to show that a Fermi level can also be defined for a solution phase. Let us suppose that the solution contains ions A^{2+} which can be reduced to A^+ by electrons coming from the solid. Equilibrium is established by the reaction



The condition for thermodynamic equilibrium is that the free energies of the reactants and products must be equal. The free energy of a charged species i is termed the electrochemical potential $\tilde{\mu}_i$ —it corresponds to the work needed to bring one mole of the species from infinity into the bulk of the phase. The equilibrium condition is therefore

$$\tilde{\mu}_{e^-} = \tilde{\mu}_{A^+} - \tilde{\mu}_{A^{2+}} \quad (2)$$

On the left hand side of the equation, μ_e , is the electrochemical potential of electrons in the solid. But this is just the Fermi energy of the electrons, E_F . The right hand side of equation (2) therefore also corresponds, conceptually at least, to a Fermi level, and Gerischer (in *Physical Chemistry IX*, A (ed. Eyring) Academic, New York, 1970) has called in the redox Fermi level, $E_{F, \text{redox}}$.

Electrochemists tend to think on a horizontal scale of relative potentials, rather than on the vertical scale of absolute free energy used in solid-state physics, but the two scales correspond in a simple way. The redox Fermi level

of a redox couple A^+/A^{2+} is related to the electrode potential⁰ $\mathcal{E}_{A^+/A^{2+}}$ on the hydrogen scale by

$${}^0E_{F, \text{redox}} = {}^0E_{F, \text{hydrogen}} - e_0 {}^0\mathcal{E}_{A^+/A^{2+}} \quad (3)$$

(e_0 is the charge on the electron)

${}^0E_{F, \text{hydrogen}}$ is the Fermi energy of the hydrogen electrode and has been estimated by non-thermodynamic methods to be about -4.3 eV (Trasatti *J. electroanal. Chem.* **52**, 313; 1974).

Once the important step of thinking in terms of Fermi energies rather than electrode potentials has been taken, the vocabulary of solid-state physics can be used without modification, and the semiconductor-liquid junction can be thought of as being entirely analogous to a more conventional junction between solids. The redox Fermi level also depends on the activity of the ions in the solution since it is easily shown that

$$E_{F, \text{redox}} = {}^0E_{F, \text{redox}} + kT \ln(a_{\text{red}}/a_{\text{ox}}) \quad (4)$$

If the Fermi level is lower in the solution than in the semiconductor before the two phases are brought into contact, electrons will spill over into the solution and reduce A^{2+} (reaction 1). A positive space charge region consisting of ionised donor states will be formed (if the semiconductor is n-type). The properties of this space charge region are sensitive to changes in the solution Fermi level and this in essence is the basis of one of the CSSDs described by Bergveld *et al.*

The ability to determine the space-charge in a semiconductor by careful selection of the ionic components of a solution contact has important practical applications apart from the CSSD. If a positive space-charge is formed in an n-type semiconductor, electrons and holes produced by illumination will be pulled apart by the strong electric field to produce a photocurrent through an external load. All we need to complete the circuit is a second electrode which can return electrons to the solution by reducing the oxidised partner of the redox couple. This kind of cell has been called a regenerative electrochemical solar cell (Gerischer *J. electroanal. Chem.* **58**, 263; 1975), since there is no net chemical change in the solution phase.

The EEC and the United States both have energy programmes in which considerable resources are being allocated to research into photovoltaic devices, including electrochemical solar cells. Last year two conferences highlighted the interest in this new approach to solar energy conversion (*Semiconductor Liquid-Junction Solar Cells*, Airlie, Virginia, 1977, Electrochemical Society, 1977; *Photovoltaic Solar Energy Conference*, Luxembourg, 1977,

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Reidel, 1978), and the meeting on photoelectrochemistry to be held in Cambridge this August will give an up-to-date review of the rapid progress in this new and exciting field. It looks as if 'wet' semiconductor devices are here to stay. □

Accelerator dating techniques

from Terry S. Mast

A DRAMATICALLY more sensitive method for measuring low concentrations of radioisotopes has generated a great deal of excitement in the field of radioisotope dating, and stimulated a remarkably large development effort in a short amount of time. Twenty months of development, which is expected to have substantial impact on many fields of research, including archeology, geology, oceanography, and astrophysics, were reviewed at a recent conference*.

The basic idea of the technique is really a synthesis of two old ideas. The first is the idea of counting directly the radioisotope atoms in a sample rather than waiting for them to decay (Muller *Science* **196**, 489; 1977). The potential gain in sensitivity is apparent when one realises that in a sample being dated with ^{14}C which has a typical decay rate of 1 decay per minute there are about 10^9 atoms of ^{14}C . Attempts to count these directly with mass spectrometers have been unsuccessful because the very low concentration of ^{14}C (10^{-12} to 10^{-14}) is inevitably masked by a much larger contamination of ^{14}N . The second idea, which solves the problem of the ^{14}N , is to use an accelerator as a mass spectrometer. Ionising and accelerating the sample atoms to a high energy (40–50 MeV) allows the separation of the ^{14}N and identification of the ^{14}C using the particle identification techniques of nuclear physics. The standard technique of counting the beta decays of the ^{14}C typically requires 1–10 g carbon and takes from 2–24 h to count. The new technique enables measurements to be made in 1 h or less with less than 5 to 10 mg carbon.

Physicists described designs for accelerator-detector systems for isotope dating and the results of the development tests being made. Archaeologists, geochemists and cosmochemists outlined new studies which can be made with the higher sensitivity, and how measurement reliability for existing studies could be proved.

Both cyclotrons and tandem Van de Graaff accelerators have been used

now to detect several radioisotopes at natural concentrations (Nelson *et al. Science* **198**, 507; 1977). The emphasis so far has been on ^{14}C (half life=5730 yr; used extensively in archaeology) and on ^{10}Be (half life= 1.5×10^6 yr; used for geochronology). There are at present six research groups making measurements and many others interested in starting programmes.

Groups at the University of California Lawrence Berkeley Laboratory and the Laboratory Rene Bernas, Orsay, have used cyclotrons. The samples are ionised in a Penning Ion Gauge source and accelerated. The cyclotron resonance condition sharply defines the charge: mass ratio accepted, and only ions with almost identical masses are accelerated—for example, ^{14}C and ^{14}N or ^{10}Be and ^{10}B . The radioisotope and stable isotope are then cleanly separated by their different ranges in an absorber. As the contaminating stable isotope is typically 10^{10} times more abundant in the beam than the radioisotope, the separation must be and is extremely efficient.

Tandem accelerators are being used by a large collaboration between the University of Rochester, the University of Toronto, and the General Ionex Corporation (Massachusetts): by a group at Chalk River, Canada; by a collaboration between the Simon Fraser and McMaster's Universities; and by a group at Oxford University. The beam for the tandem work is produced by a caesium ion sputter source, which for ^{14}C dating sputters C^- from a solid carbon sample. The separation of the ^{14}N comes from the fact that nitrogen only forms metastable negative ions which decay before passing through the tandem accelerator. A number of other contaminating isotopes, which are accelerated through the tandem, as well as the residual ^{14}N , are then cleanly separated from the ^{14}C by magnetic analysis and an ion identification detector telescope.

All groups have successfully solved the problems of detecting the radioisotopes cleanly separated from contaminating stable isotopes and with a sensitivity sufficient to measure the very low concentrations found in nature. The future development work will focus on accurate normalisation schemes, proof of reliable accuracy, and finally improvement of precision.

The main benefit of the improved sensitivity will be the ability to determine the isotope concentration with only a few milligrams of sample. This will allow the dating of many objects

which are too small to date with decay counting. Perhaps more important will be the ability to select components of a sample (such as particular proteins) which are specific to the object being dated, thus reliably removing other sources of carbon that might have leached into the object. Finally, the ability to measure several samples from the same artefact will enable checks on contamination removal procedures to be made.

The development work has so far been done on a few radioisotopes— ^{14}C , ^{10}Be and ^{36}Cl . Participants at the conference emphasised that a large number of other radioisotopes have been used in geochronology and cosmochemistry and that the new method will be useful in extending that research as well. □

Crystal defects and melting

from Robert W. Cahn

OF all the common properties of crystals, melting has a claim to be considered the most mysterious. Although there is no dearth of hypotheses, we do not know with certainty what structural features different crystal species have in common at the instant of melting.

In recent years, two venerable but mutually incompatible theories have been taken out and dusted, and their current champions each claim to have established strong evidence in their respective favour. The first is the dislocation theory of melting, first propounded by J. K. Mackenzie and N. F. Mott in 1952. The basic idea is that the energy of a dislocation array is a function both of dislocation concentration and of temperature (if dislocations are close together they partly relieve each other's stress fields), and if the temperature is high enough then a sudden catastrophic increase in dislocation density is predicted, to the point at which all crystallinity is lost. The early work is summarised by A. R. Ubbelohde (*Melting and Crystal Structure*, Oxford University Press, 1965).

R. J. M. Cotterill, W. D. Kristensen and E. J. Jensen have carried out extensive computer simulations, in two dimensions, which have shown that as kinetic energy is progressively injected, increasing populations of dislocation loops are created, grow and collapse again. When the instantaneous population grows large enough, the expanding loops "literally cut the crystal

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*Held at the University of Rochester, New York, from 20–21 April.

structure to pieces"; point defects are created in large numbers where moving dislocations intersect, and the combination of liquid-like disorder at dislocation cores and large point defect concentrations destroys crystalline order at a sharply defined effective temperature. The most recent account of these authors' work is in *Phil. Mag.* **30**, 245; 1974, and Cotterill has related this model to a number of other models of melting in a paper contributed to the Third Nordic High Temperature Symposium (1972).

The chief defect of this simulation approach is that it does not enable one to rationalise (let alone predict) the melting temperature at all accurately, although the model has been shown to be geometrically consistent with Lindemann's Rule—that at the melting point the root mean square dynamical displacement of atoms reaches a critical value: Cotterill *et al.* have shown that at a certain displacement, dislocation loops should spontaneously form. In this sense, an approximation to a melting criterion has been achieved, and to the extent that Lindemann's Rule is valid, the empirical facts are consistent with the dislocation theory in its newest variant. The model does to a first approximation interpret the volume change on melting in terms of the density changes at a dislocation core (*Phil. Mag.* **30**, 229; 1974), and latent heat can also be interpreted (*Phil. Mag.* **27**, 623; 1973).

An entirely distinct interpretation of melting, empirically beautifully self-consistent but devoid of mechanistic interpretation, is the vacancy model for metals, first advanced nearly 50 years ago by Frenkel and espoused recently by T. Górecki. In a series of papers, in English and Polish, he has adduced very extensive evidence that many different metals melt when the equilibrium vacancy concentration reaches a critical value of 0.37% and that there is then a sudden creation of further vacancies (or 'holes' in Frenkel's terms) to about 10%, at the cost of latent heat. The self-consistency of the model is tested in a number of ways. In an early paper (*Z. Metallkde* **65**, 426; 1974) Górecki has shown how changes in various physical properties on melting can be rationalised: for instance, the increase in electrical resistivity on melting is proportional to the increase in resistivity per mole of vacancies for a range of metals, and the latent heat relates as expected to the formation energy of vacancies, E_v .

In another paper (*Z. Metallkde* **67**, 269; 1976) he has shown from a consideration of known radii of first coordination spheres in solid and molten metals, that the hypothesis of 9–10% vacancies in the melt at the

melting temperature is tenable. A particularly interesting treatment *Z. Metallkde* **68**, 231; 1977) shows that the initial slope of the plot of melting temperature against hydrostatic pressure can be quantitatively interpreted in terms of the pressure dependence of the formation energy E_v . This is a particularly convincing argument for self-consistency of the model.

In a very recent paper, (*Scripta Met.* **11**, 1051; 1977) Górecki answers some criticisms and also points to recent Russian work on several metals which shows that gamma irradiation (which of course creates point defects) lowers the melting temperature by an amount which is proportional to the dose (and therefore by implication to the additional vacancy concentration). In further papers, to appear in *Z. Metallkde*, Górecki quantitatively interprets changes in thermal conductivity and surface energy on melting in terms of the vacancy model.

As an empirical exercise, Górecki's model is watertight: he has built up a very wide range of distinct phenomena which it can interpret. What it lacks is a mechanism: he nowhere explains why and how the vacancy concentration on melting increases from 0.37% to about 10%. The dislocation model offered a mechanism to account for the volume change, but that model is not (on the face of it) consistent with the observed fact that all metals melt at about the same vacancy concentration.

A model might conceivably emerge from the consideration that vacancies are believed to become more diffuse as a metal is heated—that is, an isolated vacancy at room temperature becomes a region of reduced atomic density near the melting point. This was suggested by the classical simulation experiment of Fukushima & Ookawa (*J. phys. Soc. Japan* **10**, 970; 1955): they vibrated a liquid bath on which floated a 'crystalline' raft of identical soap bubbles, and the vacancies became steadily more diffuse as the effective temperature increased. Groups of initially isolated vacancies merged into indistinguishable low density regions. The bubble raft is a very imperfect analogue of a three-dimensional crystal (though the raft did 'melt' when vibrated hard enough), but the motion of diffuse vacancy groups suggests regions of locally anomalously high vibrational amplitude, which might in turn trigger the destruction of crystalline order at a particular vacancy density. This might prove to be one way of answering the objection that a vacancy concentration of 0.37% is too low in itself to affect the stability of a crystal lattice. But that would still not provide any clue to the physical factors that determine

the increase in vacancy (or hole) concentration in the melt itself.

A halfway position between metallic crystal and melt is occupied by a metallic glass. Such glasses contain holes just as do liquids, and a recent theoretical study (Ramachandrarao, Cantor & Cahn *J. Mater. Sci.* **12**, 2488; 1977) has established a way to estimate sizes and concentrations of such holes in different metallic glasses. Such glasses are found to have a total 'free volume' at the glass transition, T_g , which is a function of hole formation energy, E_h ; but as the ratio of free volume at T_g to that at T_m is constant, hole size also varies with E_h and the vacancy concentration at T_m is constant, it would seem to follow that the hole concentration (as distinct from free volume at T_g is approximately constant. This would seem to establish a parallelism between glass transition and melting in terms of the vacancy model, but what has just been said also points to a possible weakness in Górecki's model, in that he tacitly assumes that holes in a molten metal are of the same size and have the same physical characteristics as vacancies in the corresponding solid; this approximation may well be invalid. □



A hundred years ago

AT p. 104 of this volume we called attention to an additional exception to one of Fermat's remarkable statements regarding the forms of primes. The discoverer, M. Pervouchine, has lately succeeded in showing that

$$2^{2^3} + 1$$

(a number containing many more than two millions and a half of places of figures) is divisible by the prime number

$$167,772,161 \\ 5 \cdot 2^{25} + 1.$$

or

This result has been verified by Zolotareff of the St. Petersburg Academy of Sciences. We are not told what method he employed, but it is obviously reduced to a question of mere labour by the use of the binary scale. And even this labour may be dispensed with by the aid of very simple machinery. It is much more difficult to see how M. Pervouchine was led to choose this divisor, though it would appear that the divisor was probably first assumed and the dividend calculated from it.

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review article

Baryon symmetric big bang cosmology

F. W. Stecker*

The framework of baryon symmetric big bang (BSBB) cosmology offers our greatest potential for deducing the evolution of the Universe because its physical laws and processes have the minimum number of arbitrary assumptions about initial conditions in the big-bang. In addition, it offers the possibility of explaining the photon-baryon ratio in the Universe and how galaxies and galaxy clusters are formed. BSBB cosmology also provides the only acceptable explanation at present for the origin of the cosmic γ -ray background radiation.

THE combined principles of quantum theory and special relativity, two cornerstones of modern physics, imply that the creation of matter must occur simultaneously with the creation of antimatter. This is true in the same way that the construction of a closed curve divides a simply connected surface into an inside and an outside. As is usual in astrophysics, it is both natural and logical to extend this principle of theoretical and laboratory physics by extrapolation to all other parts of the Universe to construct a coherent and rational picture of the Universe and its development. This motivation, among others, leads to the consideration of the matter-antimatter symmetric (or baryon symmetric) cosmologies which are discussed here.

On the other hand, there is no direct evidence for the existence of antimatter on a cosmic scale. (Possible indirect evidence is discussed later.) Except for the possible detection of one candidate antinucleus in the cosmic radiation¹, only upper limits exist from experiments to detect the existence of antimatter in the cosmic radiation. These upper limits seem to rule out the existence of large-scale amounts of antimatter at least within about 10^3 – 10^4 light-yr of the Sun's position in the Galaxy, given the evidence that the overwhelming bulk of the cosmic-radiation is galactic in origin^{2–4}. These limits are also consistent with a possible small extragalactic flux of cosmic-rays⁵ which may have a substantial antimatter component. Observations of the amount of cosmic galactic and background γ -radiation^{6,6} as compared with expected fluxes from the annihilation of matter and antimatter indicate that matter and antimatter, if they exist in equal amounts on a universal scale, are most probably separated into domains of the scale of clusters of galaxies. The generation and evolution of such a domain structure thus becomes an important aspect of a baryon symmetric cosmology^{7,9,10}.

Hence we come to one of the key questions of cosmology—how do we reconcile the symmetry of the matter-antimatter creation process with the apparent lack of evidence for antimatter on a large scale, at least in our corner of the Universe? Possible indirect evidence for the existence of antimatter on a cosmic scale must also be considered in this context as well as other aspects of baryon symmetric cosmology^{11,12}.

Universal versus local matter-antimatter asymmetry and domain structure

There are basically three ways of reconciling the physics of symmetric particle production processes with the lack of matter-

antimatter symmetry on a local astronomical scale. (1) One may postulate that there was, at the beginning of the big bang, a global or universal matter-antimatter asymmetry. This imbalance amounted initially to an excess of matter over antimatter by a very small, but nonvanishing amount of roughly 1 part in 10^9 . Then, in the early, dense stages of the big bang, all the matter and antimatter annihilated with the exception of the matter excess which became the present matter content of the Universe. The radiation resulting from the annihilation was cooled as the Universe expanded and is now observed as the 3-K microwave blackbody radiation. This sequence—the partially symmetric big bang (PSBB)—is the view most widely held at present. It relegates all symmetry problems to a postulated initial condition outside the context of everyday physics. (This may be analogous, in the metaphor of a curve dividing a surface, to a curve on the surface of a torus which can be constructed so that there is no inside and outside, this because of the global properties of the system.)

(2) There existed, as an initial condition, localised regions or domains in which there was an initial excess of matter over antimatter and other domains in which there was an excess of antimatter over matter⁹. These domains would have to have been too large to have been produced by statistical fluctuations or else total annihilation would have subsequently occurred as the Universe evolved^{13,14}.

(3) The Universe started out in an extremely dense, very high temperature state in a homogeneous condition with zero net baryon number (matter-antimatter symmetry). Such a state would be naturally arrived at, for example, in theoretical models where matter is created in particle-antiparticle pairs from fluctuations in the space-time metric of the Universe when it was in this compact state (see, refs 15, 16 and refs therein). One then attempts to describe processes in which the Universe evolves in a way consistent with, and dictated by, the laws of physics at extreme energies (temperatures) and densities followed by further evolution at lower temperatures and densities, until a global or domain structure is arrived at which is in accord with observational cosmology.

The construction of sequence (3) is a prodigious task, indeed one which would probably require many small steps by many workers. However, this is the most philosophically satisfactory route to follow as it is the only one which offers the potential for new cosmological discoveries based on recent advances in particle physics. It offers the hope of maximising our understanding of the evolution of the Universe as a consequence of physical processes, recognising that such an evolution is a complex process as indicated by the large amount of structure

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existing in the Universe (galaxies, clusters of galaxies, and so on). It is within the spirit of Einstein regarding natural laws, that nature is subtle but not maliciously capricious or esoteric.

Also, other important cosmological problems may be resolved. Given a universe with postulated or arbitrary boundary conditions, it is difficult to understand why the 3-K microwave background seems to be so uniform over a large scale. As we look out over the sky in different directions, we observe parts of the Universe which were not in contact with each other when the interactions which thermalised this radiation ceased. They were not in contact up to that time because they were separated by distances such that the light travel time between them was greater than the age of the Universe during those epochs. The isotropy of the background radiation then becomes enigmatical unless that background was generated by natural physical processes which follow the same evolutionary track starting from an initially simple state without arbitrary initial conditions, as in sequence (3).

Another important problem concerns the evolution of structure in the Universe. Such evolution can be more easily understood in the context of a matter-antimatter domain structure evolving out of a phase transition from a homogeneous state in the early Universe^{7,10,17}.

Other baryon symmetric cosmologies

The types of cosmological evolutionary models which conform to the programme outlined in sequence (3) will be referred to here as baryon symmetric big bang BSBB cosmologies. Other types of initially baryon symmetric and unsatisfactory cosmologies have been suggested; we mention them here briefly before more extensive discussions of BSBB cosmologies.

The first suggestion was made by Goldhaber¹⁸, who proposed that initially the Universe consisted of a single particle, the 'universon' which then split into a 'cosmon' and an 'anticosmon'. Besides not accounting for the 3-K radiation (which was unknown at the time) and the lack of a basis for such a process in particle theory, this suggestion is in effect assuming an initial condition of all baryons in the Universe, because the 'anticosmon' Universe has no observable consequences.

Variants of the steady-state cosmology¹⁹, which have the general drawbacks of steady-state cosmology (such as not accounting for the 3-K background), also have other unsatisfactory aspects. Either only matter is created, or matter and antimatter are created separately in widely separated regions (violation of conservation of baryon number, that is asymmetry in the creation process) or matter and antimatter are created together in regions of high density such as galaxy nuclei. In the latter, the amount of annihilation γ -radiation expected would far exceed that observed unless the regions where creation occurs are opaque to γ -radiation²⁰. In this case, however, it is difficult to see how matter and antimatter ever underwent a large-scale separation in the first place to form the galactic nuclei in which continuous creation is postulated to be occurring.

In the Alfvén-Klein cosmology²¹, the observable Universe began as a diffuse 'metagalaxy' consisting of equal amounts of matter and antimatter in an 'ambioplasma' whose ingredients are then separated by the combined effects of gravitation and gradients in electromagnetic fields. There are many problems with this general picture. The picture requires that the metagalaxy gravitationally collapses until it reaches a critical density where the radiation pressure from annihilation causes it to bounce and expand in accord with the Hubble law. However, detailed dynamical calculations showed that for the estimated total observable mass in the Universe, gravitational forces will overwhelm annihilation pressure and this 'bounce' will never occur²². Other problems with Alfvén-Klein cosmology are, again, the lack of explanation for the 3-K background and also insufficient separation of matter and antimatter at present. In the Alfvén-Klein model, half of our Galaxy would be

antimatter and a much higher than observed γ -ray background would be produced by annihilation.

Within the context of big-bang cosmology, various asymmetric processes of particle production and decay or destruction have been suggested as a way of producing a present global baryon asymmetry. It has been argued¹⁵ that asymmetries in possible production processes^{23,24} will not necessarily lead to a global baryon asymmetry because, in thermal equilibrium in a dense high temperature initial state, the inverse processes having the opposite asymmetry will occur with equal frequency. It has been speculated that CP-violating weak decays of certain types of particles, if such postulated particles played a prominent part in the initial stage of the big bang, might produce an asymmetry of the order of magnitude (10^{-9}) needed in the PSBB cosmology²⁴.

It has been suggested that because baryon number is not necessarily conserved when matter falls down a black hole, such processes could account for a universal baryon asymmetry. However, black holes are not selective and they will invest matter and antimatter with equal voracity in a universe which is initially baryon symmetric.

Particle physics, phase transitions and domain structure in BSBB cosmology

The evolutionary programme—which the Universe should follow according to sequence (3), to arrive at a permanent matter-antimatter domain structure consistent with observational data, was first outlined by Omnes (ref. 10 and refs therein). According to this scheme, when the Universe was above a critical temperature and its density above a critical value, a phase transition occurred, creating a structure in which there are domains containing mostly matter (positive baryon number) and domains having negative net baryon number (mostly antimatter). Subsequent annihilation pressure tended to coalesce regions of like baryon number by pushing apart regions of unlike baryon number. This process is similar to the Leidenfrost effect as first discussed in the context of the Alfvén-Klein cosmology²¹. One of the important questions in BSBB cosmology is then: what kind of phase transition can occur at the highest temperatures and densities as implied by present (and future) high energy physics?

Omnes' approach has been to use theoretical and experimental information about nucleon-antinucleon scattering properties to derive a relation giving the free energy for a system of nucleons and antinucleons interacting with each other in thermal equilibrium at a given temperature and density. The relation for the free energy as a function of net baryon number is obtained using a formula derived by Dashen, *et al.*²⁵ who generalised the formalism originally given by Beth and Uhlenbeck²⁶ to express the free energy of a system of strongly interacting particles in terms of observed scattering phase shifts. Omnes' treatment then indicated that the free energy of a gas of strongly interacting nucleons and anti-nucleons is minimised above a certain critical density when a phase separation occurs. These calculations were supported by other independent work^{27,28}. A key concept of the Omnes model is that the bound states of the nucleon-antinucleon system behave like mesons with the appropriate quantum numbers. In this 'lattice gas' type model, a nucleon and an antinucleon at high enough densities such that they are both placed within a cell of size ~ 1 fermi, look like a meson. However, the number density of mesons is determined by statistical equilibrium at temperature T so that there is a statistical exclusion principle acting to keep nucleons and antinucleons from occupying the same cell. No such principle applies to like species of baryons which results in an effective repulsion for nucleons and antinucleons; in that situation a phase separation should occur^{29,30}. Furthermore, the free energy of the system per unit volume is proportional to the ratio of surface area to volume of the domains so that the domains tend to grow to minimise this ratio. The effect is

similar to the coalescence of soap bubbles³¹. The most extensive work to date on the 'Leidenfrost' coalescence process is that of Aldrovandi *et al.*³² and Aly *et al.*³³. Electromagnetic and gravitational coalescence processes^{21,34} may, of course, combine with the Leidenfrost process to provide a substantially more efficient coalescence as the Universe evolves. This may be particularly important in reconciling some aspects of BSBB with observational data. In the Omnes model¹⁰, the observed ratio is derived from the physical processes involved.

Other types of baryon-antibaryon phase transitions in the early big bang have been suggested^{35,36}. In particular, Etim *et al.*³⁶ have applied the methods used by Omnes to show that a phase transition can exist in the early Universe using the 'statistical bootstrap model' of Hagedorn³⁷⁻³⁹ where the number of hadrons increases exponentially with mass and there exists a maximum temperature. According to Etim *et al.*, this maximum temperature coincides with the critical temperature.

The development of a phase transition stemming from physical processes at high temperatures and densities, that is, the spontaneous breaking of charge conjugation symmetry in the early Universe, is a possible fertile area for future theoretical investigation using such newly developed concepts as unified gauge theories, 'instantons', 'supergravity' and quantum chromodynamics. Certainly, the full range of modern particle physics will come into play in the early Universe.

Galaxy formation in BSBB cosmology

Various workers have tried to trace the growth of the domains of matter and antimatter from the era of phase separation to the era making the decoupling of the matter and antimatter from the blackbody radiation field^{7,10,17,31-34}. This takes us to a time of the order of 10^6 – 10^7 yr, after the big bang when the cosmic plasma was almost cool enough to combine into neutral atoms. Starting at this point in the evolution of the BSBB, the question of structure and galaxy formation arises. Models of galaxy formation from 'primordial turbulence' have always been attractive as a way of accounting for galaxy formation as well as for observed parameters such as the angular momenta and spatial distribution of galaxies⁴⁰⁻⁴⁹. However, in this work, turbulence was introduced in an *ad hoc* manner and, furthermore, such turbulence is strongly damped out in the cosmic plasma because of the very high viscosity of the blackbody radiation field which remains coupled to the cosmic plasma until the neutralisation ('recombination') epoch. Several years ago, Stecker and Puget⁷ proposed a model for galaxy formation within the context of BSBB cosmology. In this model, dissipation is constantly fought by continuing radiation pressure from annihilation on the boundaries of domains which regenerates the turbulence. Radiation pressure from the annihilation, being directed generally away from the boundary regions, can drive mass fluid motions of the domains as well as causing further coalescence until the domains reach the size of galaxy clusters.

At the recombination epoch, two important changes were caused in the cosmic fluid motions. The viscosity dropped drastically and the turbulent fluid motions became supersonic. This occurred because the sound speed dropped sharply from its value in the cosmic plasma of $3^{-1/2}c$ (because the momentum was transferred by radiation) to the thermal velocity of the neutral gas. Thus, whereas the cosmic plasma behaved as a viscous incompressible fluid, both 'small-scale' turbulence and density fluctuations could start to build up in the decoupled atomic fluid and later contract to form galaxies. The Stecker-Puget model thus resembles proposals on galaxy formation dating back to von Weizsäcker⁴⁰ and Gamow⁴¹ with the significant difference that in the BSBB cosmology annihilation pressure can provide a continuous source of generating turbulence. The basic results and concepts of the Stecker-Puget model have found support in later work by Dallaporta *et al.*¹⁷.

Observational consequences of BSBB cosmology

One of the most significant consequences of BSBB cosmology lies in the production of an observable cosmic background of

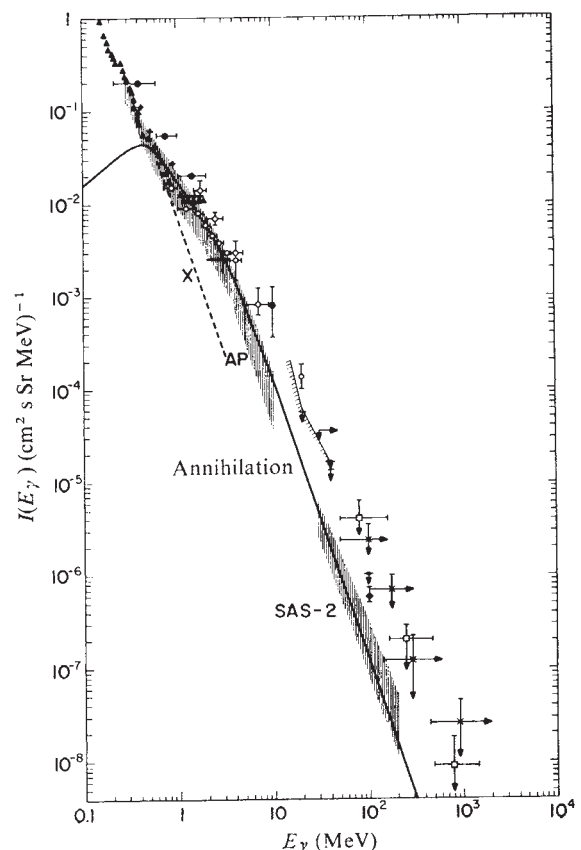
γ -radiation from the decay of π^0 -mesons produced in nucleon-antinucleon annihilations throughout the history of the Universe. This is also perhaps the most encouraging aspect of BSBB cosmology as it satisfactorily explains the observed energy spectrum of the cosmic background γ -radiation which no other proposed mechanism does^{12,50-55}.

Figure 1 shows the observational data on the γ -ray background spectrum (from refs 11, 52, 54 and 56), data of Makino⁵⁷ are not shown but they agree with other data. The dashed line is an extrapolation of the data from the X-ray range^{58,59}. The theoretical curve is the calculated annihilation spectrum adopting a mean present universal gas density of $3 \times 10^{-7} \text{ cm}^{-3}$ (ref. 53) and a Hubble constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. This corresponds to a value of $\Omega \simeq 0.1$ where Ω is the ratio of the mass of the Universe to that needed to gravitationally close the Universe. The density adopted here fits the more recent revised Apollo data⁵⁶ better than the value of 10^{-5} cm^{-3} we originally used. Interestingly, the values of H_0 and Ω used in ref. 53 are more in line with present observational evidence regarding these parameters. Gott and Turner⁵⁹ estimate that the mass in galaxies gives $\Omega_G = 0.08$ and that although $\Omega \geq \Omega_G$, $\Omega \simeq \Omega_G$.

Other attempts to account for the γ -ray background radiation above 100 MeV energy⁶⁰⁻⁶² give spectra which are inconsistent with the observations, generally by being too flat^{12,54,55}. Previous attempts to account for this radiation have also been found wanting⁵⁰⁻⁵².

Neutrinos are another annihilation product. The neutrino background spectrum in the energy range 1–50 MeV should be similar in shape and intensity to that of the background γ -radiation. However, because of the small interaction cross

Fig. 1 Theoretical matter-antimatter annihilation spectrum and observational data on the cosmic background γ -radiation. Points with arrows show upper limits from balloon data. The black diamond, representing the OSO 3 satellite data⁶ contains a probable contribution of high latitude galactic γ -rays⁵⁴. Data from the Apollo flights⁵⁶ and the SAS 2 satellite⁵⁴ are shown as shaded areas.



section of these neutrinos, their detection at present is not feasible.

Similar detection problems face the proposal of Cramer and Braithwaite⁶³, who pointed out that radiation from antimatter supernovae may show some circular polarisation opposite to that of matter supernovae.

If the detection of an antinucleus in the cosmic radiation, as has been suggested¹, could be confirmed, it would probably establish BSBB cosmology.

Another observational consequence of BSBB cosmology is the expected distortion of the 3-K microwave blackbody radiation (see ref. 64 and refs therein). An analysis of the observations of the 3-K background⁶⁵ indicates that there is a distortion at the 90% confidence level. This will be discussed further in the next section.

Problems

Two areas of particular concern have been discussed concerning BSBB cosmology—distortion of the 3-K background radiation and nucleosynthesis of helium. Two types of distortion of the 3-K background are expected to occur: (1) distortion owing to bremsstrahlung radiation of suprathermal electrons which affects the Rayleigh–Jeans part of the 3-K spectrum; and (2) distortion from Thomson scattering after the decoupling epoch which affects the Wien part of the spectrum. The Rayleigh–Jeans part of the spectrum is affected by annihilation taking place when most of the energy density in the Universe was in the form of radiation, long before neutralisation of the cosmic plasma and galaxy formation, and it is difficult to determine the precise amount of annihilation to expect at this remote epoch; it depends on the details of the coalescence process. Extrapolation of the γ -ray background data suggests that possibly as much as 99.999% of the annihilation may have taken place before the Rayleigh–Jeans distortion epoch whereas calculations⁶⁶ give a lower limit of 96.5–99% on this value. Thus, there is no critical conflict between model and observation on this point, although Ramani and Puget⁶⁷ have concluded that for the specific coalescence model of Aldrovandi *et al.*³² taken alone, there may be a conflict unless $\Omega \lesssim 0.01$.

The Wien (or high frequency) distortion from electron–photon scattering, sometimes referred to as comptonisation, has been treated elsewhere^{67–70}; in refs. 68 and 69 it is concluded that the high frequency distortion predicted by the Stecker–Puget model does not conflict with the observations. The calculations of Ramani and Puget⁶⁷, when combined with the analysis of Field and Perrenod⁶⁵, imply consistency only for $\Omega \lesssim 0.22$ (ref. 12), consistent with the value of 0.1 used to fit the γ -ray background observations. Jones and Steigman⁷⁰ indicate that there is no conflict between the coalescence model of Aldrovandi *et al.*³² and the analysis of Field and Perrenod⁶⁵, however, these authors conclude that the Stecker–Puget turbulence model leads to distortions in conflict with observation. Further analysis of subtleties not taken account of in the original work of Stecker and Puget may help resolve some of the difficulties. For one thing, in the annihilation process, photons are created and not just scattered so that at least partial thermalisation may result. This is not taken account of by Jones and Steigman. There is also the question of what percentage of annihilation energy is ‘fossilised’ in mass motion as opposed to that dissipated. On the other hand, the original Stecker–Puget model may have overestimated the role of annihilation-generated turbulence in the galaxy formation process. Although the mechanism may provide a key trigger, mass perturbations and the effects of subsequent gravitational and magnetic fields, not fully considered in the original model, may well play a prominent part. However, the overall scale of structure as given by the Stecker–Puget model, seems to be supported by a recent analysis of the spatial distribution of galaxies⁷¹.

We next consider the nucleosynthesis problem. Within the context of the Omnes coalescence model, nucleosynthesis cannot take place in the BSBB^{72,73}, because at the time nucleosynthesis would occur, the mean size of coalesced domains

would be smaller than the neutron diffusion length for escape from the domains. Thus, the neutrons would be annihilated before they can participate in the nucleosynthesis process. There are two ways around this apparent difficulty: (1) if the coalescence process is more efficient than the initial work indicated, the size of the resulting domains could be larger than the neutron diffusion length. Combes *et al.*⁷³ find that for this to be so, the domain sizes should be $\gtrsim 1.5 \times 10^8$ cm during the nucleosynthesis epoch. (2) Nucleosynthesis may not have taken place in the big bang, but rather may have taken place in ‘little bangs’ early in the life of galaxies⁷⁴. There is some support for this point of view in optical observations of a galaxy-to-galaxy variation of helium abundance⁷⁵ which would not be expected if the helium was produced uniformly in the big bang.

As already stated, the upper limits which exist on anti-nuclei in the cosmic radiation (see Ivanova *et al.*⁷⁶) do not conflict with the existence of antimatter in other galaxies in accord with BSBB cosmology.

Conclusion

Both the encouraging aspects and the problems of BSBB cosmology should be an impetus for future work. Particularly significant areas for research are (1) the implications of recent advances in particle physics for the phase transition epoch; (2) a more complete treatment of the coalescence process; and (3) a more complete treatment of the galaxy formation process. In this review, it has only been possible to touch briefly and qualitatively on these areas, however it is hoped that this will help towards an understanding of this presently unorthodox but exciting and potentially vastly rewarding field of modern astrophysical cosmology.

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articles

On recent lunar atmosphere

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Two independent kinds of lunar expedition data are considered which put forward some strong theoretical evidence of the existence of a considerable atmosphere on the Moon during the greater part of its history. The transformation of a meteoritic flux when passing through an arbitrary atmosphere has been analysed and tentative estimates of the lunar atmospheric power presented.

THE statistical mass distribution of particles of the meteoritic flux can be described by introducing either the probability function $F(m)$ or the distribution function $f(m)$. The former gives the probability that a random meteoroid has mass more than m while $f(m)dm$ is the probability that it has mass between m and $m+dm$. If one of these is known the other function can be calculated, because $f(m) = -dF/dm$ and $F(m) = \int_m^\infty f(m')dm'$ (to be precise, we shall not denote the limit and variable of integration by the same symbol).

Meteoritic mass distributions have been reliably obtained^{1,2} from crate size distributions observed on vitrified lunar samples. The peculiarities of the distribution found can be seen clearly from the so-called differential slope curve $\gamma(m)$ which is expressed using $F(m)$ and $f(m)$ by

$$\gamma(m) = -\frac{d \ln F(m)}{d \ln m} = -\frac{m}{F(m)} \frac{dF}{dm} = \frac{m}{F(m)} f(m) \quad (1)$$

Five typical curves for $\gamma(m)$ are shown in Fig. 1. The behaviour of curves 1 and 2 is opposite to that of curves 3 and 4, and curve 5 is thought to be their superimposition. The abrupt gap from 10^{-11} to 10^{-7} g was not generally observed in previous direct meteoritic studies, and as Hörz *et al.*¹ acknowledge, no reasonable explanation of this inconsistency has been put forward.

Although the total time of cratering has been measured the position of this period is uncertain, hence, there is no reason to assume that the cratering on different samples

occurred at the same time. On the other hand, the exposure times of about 10^5 yr for the samples corresponding to curves 3 and 4 are greater than those for 1 and 2, therefore, the former distribution could have formed during a period of almost complete absence of the small particles; subsequently, due to some cause small meteoroids apparently formed two curves 1 and 3 as well as the tail of distribution 5. Such a picture of the phenomena can be justified by assuming that the Moon possessed at some time a dissipating atmosphere which cut off the small meteorites.

Ablation and deceleration of a meteoroid

To investigate the proposed hypotheses quantitatively we consider the ablation and deceleration of meteoroids as they pass through the atmosphere. This problem has been investigated in detail for the Earth's atmosphere³⁻⁵ and the usual assumptions about the atmospheric structure are avoided in our approach.

Let m and v be mass and velocity of a meteoroid moving through an atmosphere which has a density $\rho(y)$ at altitude y . It is evident that $dy/dt = -v \cos \theta$ where θ is an angle of incidence. The conventional³ equations of motion and loss of mass are

$$\frac{dv}{dt} = -\eta m^{-1/3} \rho(y) v^2; \quad \frac{dm}{dt} = -\beta m^{2/3} \rho(y) v^3 \quad (2)$$

The constants η and β can be expressed in terms of drag coefficient Γ , heat-transfer coefficient Λ and coefficient of shape A , as follows³⁻⁵

$$\eta = \Gamma A \rho_m^{-2/3}; \quad \beta = \frac{\Lambda A}{2Q} \rho_m^{-2/3}$$

where ρ_m and Q are, respectively, the density and the heat of vaporisation for the meteorite material.

In order to estimate η and β , it is convenient to assume $\Gamma = \Lambda = 3/4$, $A = (9\pi/16)^{1/3} \approx 1.21$ and according to Öpik³, $\rho_m = 2.5 \text{ g cm}^{-3}$ and $Q = 8 \times 10^{12} \text{ erg g}^{-1}$. This gives $2\eta = 1 \text{ (cm}^2 \text{ g}^{-2/3})$ and $6\eta/\beta = 10^{12} \text{ cm}^2 \text{ s}^{-2}$, hence, the value $v_0 =$

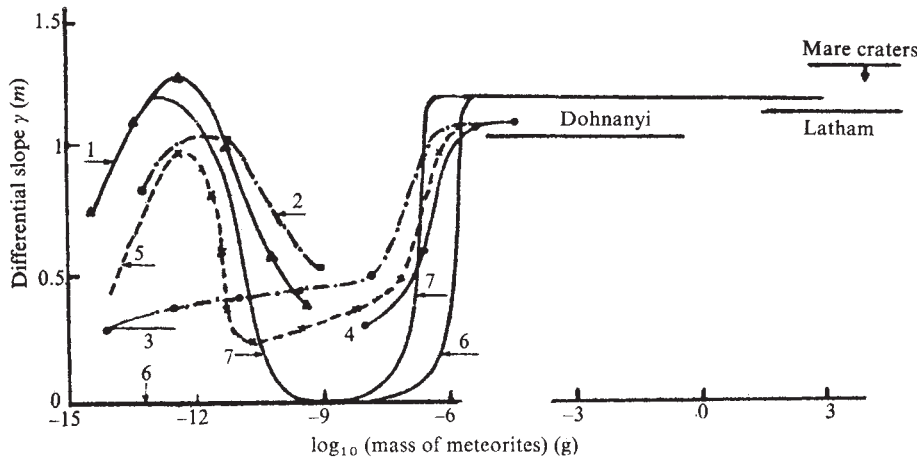


Fig. 1 The differential slope $\gamma(m)$ defined by equation (1) as a function of meteorite mass (m). The sample numbers corresponding to curves 1 to 5 are: 1, (▲) 15078; 2, (■) 15017; 3, (—●—) 15286; 4, (—●—) 12064; 5, (×) 15206; for details see ref. 1. Curves 6 and 7 represent the theoretical function $\gamma(m)$ corresponding to the flux $f_i(m)$ cut off by the atmosphere with $z_t = 0.7 \times 10^{-2}$ and $z_t = 0.3 \times 10^{-2} \text{ g cm}^{-2}$ respectively. The last curve also involves the existing flux. The position m_B of the sharp bend in theoretical curves is determined by $\log m_B = 31g(z_{te})$.

$(6\eta/\beta)^{1/2} = 10^6 \text{ cm s}^{-1}$ can be chosen as a unit of velocity. Then we introduce new variables

$$z = \int_0^y \frac{\rho(y')}{\cos\theta} dy'; \quad \mu = \frac{m^{1/3}}{2\eta}; \quad u = \left(\frac{v}{v_0}\right)^2$$

such that both z and μ are measured in g cm^{-2} while u is dimensionless. Because $dz/dt = \rho(y)(dy/dt)/\cos\theta = -\rho v$ and $d/dt = (dz/dt)(d/dz)$ we have $d/dt = \rho(y)v(d/dz)$. Thus, using new variables reduces equation (2) to

$$\frac{du}{dz} = \frac{u}{\mu}; \quad \frac{d\mu}{dz} = u \quad (3)$$

It follows that near the surface ($y = 0$) the final 'size' μ_f and 'velocity' u_f of the meteoroid, for which the initial values of the same quantities were μ_i and u_i , are determined implicitly by

$$\mu_f = \mu_i \exp(u_f - u_i), \quad \int_{u_f}^{u_i} \exp(u) \frac{du}{u} = \frac{z_f}{\mu_i} \exp(u_i) \quad (4)$$

where $z_f = z(\infty)$ is the total mass of the atmosphere within the column of unit area (we assume $\cos\theta = 1$).

It can be shown that meteoroids with 'size' $\mu_i < z_f u_i$ are decelerated rapidly and equation (4) is simplified to

$$\mu_f = \mu_i \exp(-u_i); \quad u_f = \exp\left\{-c - \frac{\exp u_i}{u_i} \left(\frac{z_f u_i}{\mu_i} - 1\right)\right\}; \quad c = 0.577 \quad (5)$$

In an opposite case when $\mu_i > z_f u_i$ we have

$$u_f = u_i + \ln\left(1 - \frac{z_f u_i}{\mu_i}\right); \quad \mu_f = \mu_i - z_f u_i \quad (6)$$

Thus, the quantity $z_f u_i$ gives a boundary value between mass regions of rapid and slow deceleration.

Transformation of statistical distributions

To study how the meteoritic flux affects the surface of a planet, it is necessary to find its mass and velocity distribution function $f(\mu, u, z)$ corresponding to the 'altitude' z and then put $z = 0$

(the surface). When a meteoroid is passing through an atmosphere its varying values of μ and u describe a path on the plane (μ, u) . The path starts at the point (μ_i, u_i) and finishes at (μ_f, u_f) . Because the motion along this path of the point corresponding to a given particle obeys equation (3) and hence is not random, the probability that the point remains all the time within the path is unity. Therefore $df = 0$ or $(\partial f/\partial z)dz + (\partial f/\partial \mu)d\mu + (\partial f/\partial u)du = 0$ along the path, which implies $dz, d\mu$ and du satisfy equation (3). It follows that $f(\mu, u, z)$ obeys the partial equation

$$\frac{\partial f}{\partial z} + u \frac{\partial f}{\partial \mu} + \frac{\mu}{z} \frac{\partial f}{\partial u} = 0 \quad (7)$$

which is the simplest example of so-called Liouville's equation⁶.

Equations (5) and (6) give explicit (approximate) expressions for the final values of μ_f and u_f in terms of μ_i and u_i . By analogy, it is possible to express u_i and μ_i from equation (4) as explicit functions of μ_f and u_f . Let $u_i = \varphi(\mu_f, u_f)$ and $\mu_i = \psi(\mu_f, u_f)$ be these exact functions (they can be found from equation (4) either numerically or graphically or by infinite expansions). Then the solution of equation (7) that is, the required distribution function $f_i(\mu_f, u_f) = f(\mu_f, u_f, 0)$ corresponding to the initial $f_i(\mu_i, u_i)$ can be written in the form

$$f(\mu_f, u_f) = f(\varphi(\mu_f, u_f), \psi(\mu_f, u_f)) \quad (8)$$

whatever the initial distribution, this points to μ_f and u_f being strongly correlated, which implies that at the surface, according to equations (5)–(8), all the small particles are predominantly slow. Therefore, to find the mass distribution of high velocity meteorites ($v_f > 10^6 \text{ cm s}^{-1}$ or $u_f > 1$), which were considered in refs 1 and 2 and Fig. 1, we should sum $f_i(\mu_f, u_f)$ over all $u_f > 1$ and write

$$f_i(\mu) = \int_{u_f > 1} f_i(\mu, u_f) du_f \quad (9)$$

The standard way to calculate such integrals is by numerical computation, but I had to develop a special analytical technique. The result representing the size distribution function $f_i(\mu)$ for all the fast meteorites reaching the planet surface is shown in Fig. 2 by curve 2. The initial velocity distribution has been assumed to be rectangular but the mass distribution function $f_i(m)$ was taken to be of the form $f_i(m) = \text{const. } m^{-(1+\gamma)}$ at $\gamma = 1.2$. The plot has been made in universal coordinates so that it can be applied to an arbitrary planet atmosphere and it is sufficient to fix z_f corresponding to a given atmospheric mass to turn the m/z scale into μ . In other words, one can

pass from one atmosphere (or z_f) to another by shifting the curve along the abscissa. It is also important to note that the position μ_B of the maximum is determined by condition $\mu_B/z_f = e$.

As already mentioned, having obtained $f_r(\mu)$, one can calculate the corresponding function $F_r(\mu)$ and then $\gamma(\mu)$, returning finally to the observed data. Theoretical results for $\gamma(m)$ are shown in Fig. 1. The 6th curve corresponds to $z_f = 0.7 \times 10^{-2} \text{ g cm}^{-2}$. Curve 7 gives $\gamma(m)$ for a superimposition of the flux cut off by the atmosphere with $z_f = 0.3 \times 10^{-2} \text{ g cm}^{-2}$, and the initial flux where the ratio of exposure time of the former flux to that of the latter was taken to be 10^6 . The superimposition corresponds to a sample that had first been bombarded in the atmosphere, was then buried and again appeared on the surface under the existing meteoritic flux. Such a sample history is typical because of the permanent 'gardening' of the regolith by meteoroids.

Bearing in mind that Hörz *et al.*¹ used an average impact velocity—the correlation was ignored—then curves 6 and 7 (for which only initial distributions were uncorrelated, in contrast to the final ones corresponding to these curves) are in good agreement with the data. If one allows for the $v-m$ correlation, the data in Fig. 1 should move closer to the theoretical curves.

Lunar regolith granularity

Independent and more convincing evidence^{8,11} for the existence of the atmosphere on the Moon is that the regolith is relatively depleted of small particles and that the depletion increases with increasing depth. Conventional concepts⁶⁻¹¹ about the regolith being formed mainly by meteoritic bombardment are quantitatively inconsistent, unless the small particles in the meteoritic flux are assumed to be cut off in the past. In other words, the existing meteoritic flux contains too few small particles to produce the observed regolith granularity.

To analyse this problem the concept of the mass share $F_s(M)$ is introduced. For a sample of soil we define $F_s(M)$ as a ratio of the summed mass of all particles having masses

greater than M to the mass of the sample. Let us estimate the upper limit for $F_s(M)$ of the regolith formed by a meteoritic flux with certain mass distribution function $f(m)$. For this we assume that meteorites act solely on rocks, that is, the meteoritic rework of the regolith is neglected, to obtain the distribution for the very immature soil and, therefore, the real granularity should be finer than the theoretical one.

Hence⁹ let $g(M/m)$ be a mass share (analogous to $F_s(M)$) of rock fragments with masses greater than M , produced by an impact of the fast ($u > 1$) meteorite with mass m , and let $M_{\text{tot}}(m) = Km$ be their total mass. (According to ref 12, K is independent of m whereas K does not need to depend on u because the final expressions do not involve K .) Then the mass of fragments having masses from M to $M+dM$ is $M_{\text{tot}}(m) \frac{\partial g(M/m)}{\partial M} dM$.

If $M_{\text{max}}(m) = Bm$ (B depends slightly on u (ref. 12)), is the mass of the maximal fragment, then $g(M/m) \Big|_{M > M_{\text{max}}} = 1$

and $\frac{\partial g}{\partial M} \Big|_{M > M_{\text{max}}} = 0$. Therefore, the mass of fragments with masses M to $M+dM$ which is formed by the whole meteoritic flux with the distribution $f(m)$ should be proportional to

$$dM \int_0^\infty f(m) M_{\text{tot}}(m) \frac{\partial g(M/m)}{\partial M} dm = K \cdot dM \int_{M/B}^\infty m f(m) \frac{\partial g(M/m)}{\partial M} dm \quad (10)$$

To determine the observable mass share $F_s(M)$ of soil particles with masses not less than M we should integrate the last expression over masses between the limits M and ∞ and then normalise the result by the standard condition $F_s(0) = 1$. Hence one obtains

$$F_s(M) = \frac{\int_0^\infty f(m) m [1 - g(M/m)] dm}{\int_0^\infty f(m) m dm} = \quad (11)$$

$$\frac{1}{\langle m \rangle} \int_{M/B}^\infty f(m) m [1 - g(M/m)] dm$$

Here we denoted the denominator of the second expression by $\langle m \rangle$. As $f(m)dm$ is the relative amount of meteorites with masses from m to $m+dm$, $\langle m \rangle$ is just the mean meteoritic mass.

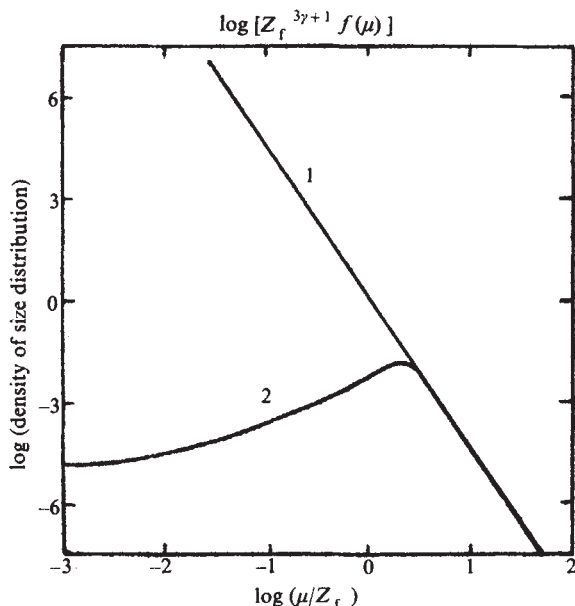
We will use an empirical¹² function $g(M/m) =$

$$\left(\frac{M}{M_{\text{max}}} \right)^{\alpha/3} = \left(\frac{M}{Bm} \right)^{\alpha/3}$$

with $0.3 \lesssim \alpha \lesssim 0.6$ and $B = 2.5 \times 10^3$. In cases when the regolith is produced by the existing flux, we incorporate into equation (11) the distribution $f(m) = f_i(m)$ written in the conventional form

$$f_i(m) = \frac{\gamma}{m_0} \left(\frac{m_0}{m} \right)^{1+\gamma}$$

Fig. 2 The universal law of the meteoritic size-distribution transformation due to the passage of fast meteorites through the atmosphere. Curve 1 represents the initial and curve 2 the final distribution functions. The universal character of the curve results from the invariance of equation (4) under transformation $z \rightarrow az$ and $\mu \rightarrow a\mu$ where $a = \text{constant}$. Due to the log-scale both curves are indiscernible in the region of large masses.



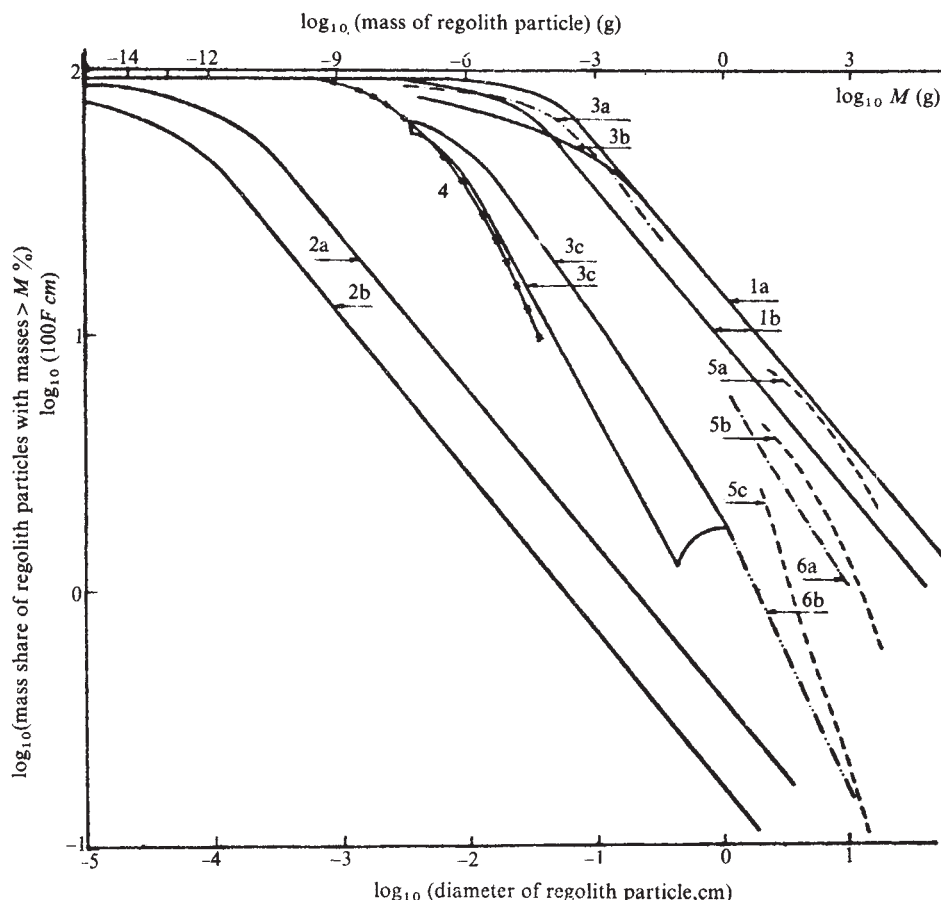


Fig. 3 The mass (size) distribution $F_s(M)$ of regolith particles. Curves 1a, 1b represent the upper estimates corresponding to $f(m) = f_i(m)$, and curves 2a, 2b to $f(m) = f_t(m)$. The straight line parts of curves 1a, 1b coincide with those calculated by formulae obtained in ref. 12. Theoretical curves, 1a, $z_f = 1.3 \times 10^{-2} \text{ g s}^{-1} \text{ m}^{-2}$; 1b, $z_f = 0.7 \times 10^{-2} \text{ g s}^{-1} \text{ m}^{-2}$; theoretical curves, 2a, $\alpha = 0.6, m_0 = 10^{-13} \text{ g}$; 2b, $\alpha = 0.4, m_0 = 10^{-14} \text{ g}$; 3, Apollo 11 and 12 samples 10084 and 12001 (ref. 9); 5, Lunokhods 1 and 2 (ref. 10); 6, Surveyors⁸, 6a, Surveyor 3; 6b, Surveyor 5.

at $m \geq m_0$ and $f_i(m) = 0$ at $m < m_0$ and find

$$F_s(M) = \begin{cases} \frac{1-3(\gamma-1)}{3(\gamma-1)+\alpha} \left(\frac{M}{M_0} \right)^{\alpha/3}, & \text{at } M \leq M_0 \\ \frac{\alpha}{3(\gamma-1)+\alpha} \left(\frac{M_0}{M} \right)^{\gamma-1}, & \text{at } M \geq M_0 \end{cases} \quad (12)$$

Here $M_0 = Bm_0$ is the mass of the maximal fragment formed by the minimal meteorite with mass m_0 (below we put $m_0 = 10^{-12} \text{ g}$ or $m_0 = 10^{-13} \text{ g}$ for estimations).

The results following from equation (12) for $\gamma = 1.2$ are represented in Fig. 3 by curves 2a (corresponding to $m_0 = 10^{-12} \text{ g}$ and $\alpha = 0.6$) and 2b ($m_0 = 10^{-14} \text{ g}$ and $\alpha = 0.4$). The major outcome is that the curves 2 and 2a are well below the data points, although by the new calculation they should have been the upper limits if the meteoritic flux had actually possessed the current mass distribution. According to equation (12) the (same) slope of straight line parts of curves 2a and 2b is equal either to $\gamma-1$ if one uses the mass scale or, to $3(\gamma-1)$ if the size scale is used. The close resemblance of the slopes of the experimental and theoretical curves occurring in the region of large masses permits us to conclude that the regolith was indeed formed by meteorites.

We can now bring these curves into agreement by assuming that small masses of the bombarding flux were cut off, and substituting for $f(m)$ in equation (11) the distributions $f_i(m)$ corresponding to curves 6 and 7 in Fig. 1. The results, curves 1 and 1a in Fig. 3, agree quite well with those corresponding to the most coarse-grained samples and, hence, can really be considered as the upper limits in question.

Thus, the value $z_f \approx 10^{-2} \text{ g cm}^{-2} \text{ s}^{-1}$ was reflected in both the vitrified samples and the regolith granularity. Therefore, we suggest that $z_f \approx 10^{-2} \text{ g cm}^{-2}$ is the most reasonable estimate for the lunar atmospheric power during a certain period.

Conclusion

Both kinds of data considered, in principle, are dated from a sound statistical (correlation) analysis, but we can only assume that $z_f \approx 10^{-2} \text{ g cm}^{-2}$ was true no more than 100 Myr ago.

We have implied here that the behaviour of the meteoritic mass distribution was constant throughout the Solar System. Several arguments both for and against this assumption have been discussed although the latter can be readily interpreted through our approach. Moreover, much more rigorous substantiation of the assumption is that the Solar System took between 10 and 100 Myr (refs 13, 14) to form, and the time of the earlier period after which the flux should change is much slower. Furthermore, direct estimates¹⁴ of the collision probabilities also show that the existing flux could not have appreciably changed during the past 100 Myr. Thus, the assumption about the existence of the lunar atmosphere is undoubtedly less radical than the assumption of abrupt changes in the meteoritic mass distribution, although both points of view should lead to reconsideration of planetological concepts.

It is important that if the vitrified samples were horizontal under bombardment in the atmosphere (a probable position) then the probability of oblique incidences should be lower than that of the normal ones. The significant abundance of normal incidence craters was observed in refs 1 and 2. Hence, the proper analysis of correlations between incidence angles and particle masses can also elucidate the problem quantitatively.

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Thermal alteration of Cretaceous black shale by basaltic intrusions in the Eastern Atlantic

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DSDP site 41-368 in the eastern Atlantic represents an unusual locality, where the behaviour of a short column of organic-rich shale can be studied in relation to the steep thermal gradient imposed by basaltic intrusions. This study indicates that the dominant effects on the measured parameters of the organic matter were thermal and that compositional variations in the original matter were minor in comparison.

ORGANIC-RICH, black shales of Cretaceous age have been recovered from various Deep Sea Drilling Project sites in the Atlantic Ocean¹⁻⁴. Some of these black shales closely resemble the Cretaceous La Luna formation⁵⁻⁷, which is the presumed source rock of the northern Venezuelan petroleum from the Maracaibo Basin. At site DSDP 41-368 the black shale was intruded by diabase sills and small quantities of lightweight extractable hydrocarbons were reported³. Geochemical analyses across these sill intrusions presented an excellent *in situ* thermal experiment to monitor the effects resulting from the expulsion of volatile organic components.

Site 41-368 is located in the Cape Verde Rise (17°30.4'N, 21°21.2'W; water depth 3,367 m) (ref. 2). Turonian-Albian black shales were horizontally penetrated by two thin (~20 cm) and one thick (15 m) diabase basalt units⁸. The age of the major unit was determined by the K/Ar method to be approximately 19 Myr (early Miocene)⁹. The general lithology of this Cretaceous section from site 41-368 is shown in Fig. 1.

The samples were obtained from the working cores of the DSDP repository. All samples were freeze dried, powdered, and treated in the same manner for (1) lipid extraction, separation and gas chromatographic analyses; and (2) kerogen separation and analyses. The detailed data and procedures will be published elsewhere¹⁰.

The sample descriptions and analytical results are given in Table 1, where the data (samples 1-15) are listed sequentially with increasing depth. In addition, two composite samples of unaltered shale are also included (samples 16 and 17). Samples 18 and 19 are data from the same core quoted for comparison purposes from Deroo *et al.*¹¹. The sample positions are indicated in the lithologic column of Fig. 1 by circled numerals.

The sediments in the proximity of the sills were baked

and between the sills (for example, samples 4-8) they appear to be carbonised as well. Samples 9 and 10 are light grey in colour and partially fused indicating that the organic matter has been expelled. Samples 4-8 and the contacts with the intrusions exhibit microfissures and rusty areas, indicating mineralisation and alteration due to circulation of hydrothermal fluids.

The organic carbon values vary, but are generally lower in the proximity of the intrusions (Table 1). Sample 3, located 20 cm above the upper sill, contained 38% carbonate, indicating that its thermal stress did not exceed about 900 °C, the temperature of decomposition of CaCO₃. Significant carbonate values are also observed for samples 8, 12 and 15. Sample 8 is located about 80 cm above the major sill and appears heavily carbonised. Samples 12 to 15 are below the major sill and appear unaltered by the intrusion. Elemental sulphur is present in large amounts in the extracts from samples 4-10 in the proximity and between the sills. This sulphur seems to be derived from the disproportionation of pyrite.

Lipids

Based on the lipid data, samples 2, 14 and 15 resemble typical unaltered black shale as found at other DSDP sites in the Atlantic (site 41-367⁶; or leg 40³). The overall yields of total solvent soluble material (total lipids) are variable due to the content of asphaltic (in altered samples) or polar material (in unaltered samples) and in some cases some elemental sulphur. The amounts of hydrocarbons exhibit a minimum near and between the sills (compare Table 1 and Fig. 2). The data for *n*-fatty acids and ketones will be reported elsewhere¹⁰. In unaltered black shales the phytane (Ph) concentration is much greater than that of pristane (Pr) (for example, refs 5, 6, 12), reflecting the strongly anoxic palaeoenvironmental conditions of sedimentation¹³. This relationship is observed for unaltered samples above and below the sills (for example, samples 1, 2, 14-17). An increase of Pr to Ph is observed for samples 15 to 11 on approaching the sill (Table 1). Some of this Pr seems to be thermally derived. For samples 5-8 between the sills, Pr > Ph (Table 1). Both Pr and Ph contents decrease near the sills, possibly due to further cracking to smaller molecules and expulsion in the sill proximity.

The distributions of the *n*-alkanes and their concentrations in relation to that of the isoprenoids for samples 2 and 15-17 are typical of Cretaceous black shales from the Atlantic^{5,6,12}. The lower molecular weight homologues

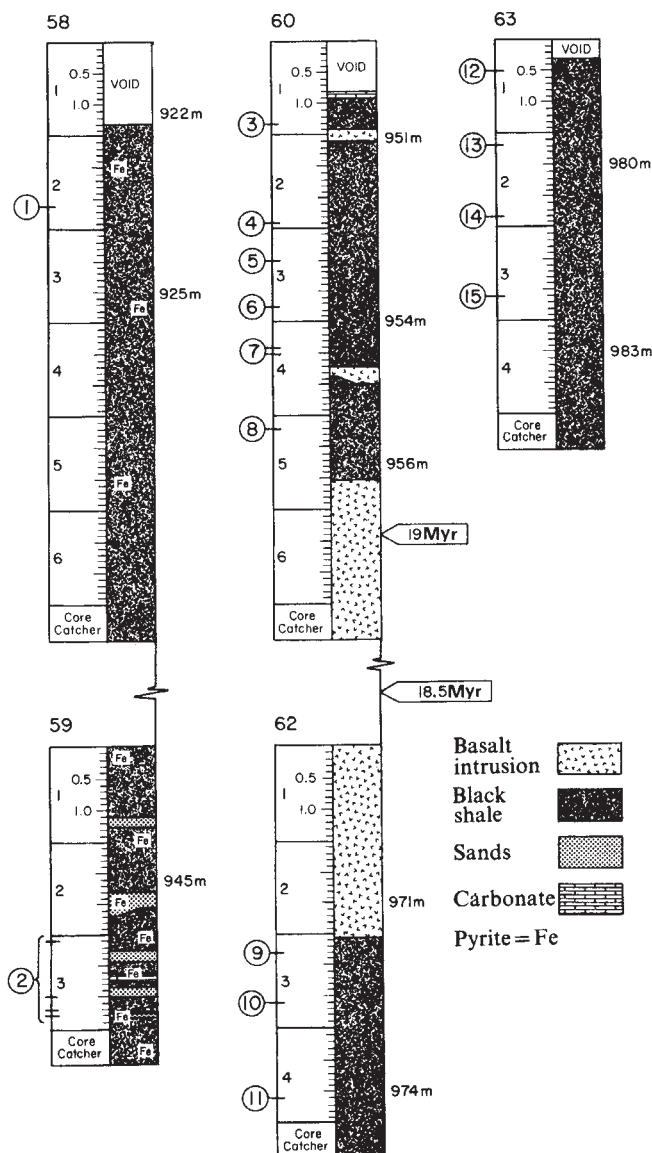


Fig. 1 Lithologic sequence for the Cretaceous shale from site 368 with the basalt intrusions. The samples that were analysed are indicated by the circled numerals.

(< n -C₂₅) exhibit essentially no carbon number predominances with maxima at n -C₁₅, C₁₇ and C₂₃. Such a distribution is attributable to an autochthonous origin from microbiota^{14,15}. The higher weight homologues (> n -C₂₅) exhibit a strong odd-to-even carbon number predominance with a maximum at n -C₂₉ or n -C₃₁, indicating a vascular plant wax origin^{14,15}. Samples 11–14 exhibit a progressive dilution of the n -alkanes by the more volatile homologues maximising at n -C₁₇. Samples 3–10, from the proximity of or between the sills, exhibit low levels of n -alkanes with narrow distributions maximising at n -C₁₇, or in some cases n -C₁₅, C₁₉ and C₂₂. The alkane distribution for sample 8 is very narrow with a slightly higher yield (180 p.p.m.) compared with its neighbours. This sample is located between the major and middle sills, which are only 1.6 m apart. We suggest that thermally derived hydrocarbons were partly trapped between the sills, possibly absorbed to the carbonised residue.

The total hydrocarbon yield is plotted against depth in Fig. 2. A maximum is observed about 7 m below the major sill (sample 10) and a relative increase is also observed about 6–8 m above the major sill (sample 3). The lower content above the major sill indicates that the expelled volatile

components may have migrated out laterally along sand (silt) or carbonate units, whereas below the sill they have remained in relatively high concentrations. The total hydrocarbon yield reaches a minimum in the proximity of the major intrusion (samples 4–7, 9 and 10).

Metalloporphyrins were isolated from samples of this core across the sills¹⁶. In this case porphyrin dealkylation occurred over transalkylation, which is a lower temperature process.

Asphaltenes seem to be present in most samples and they were separated from samples 2, 3 11 and 13. A higher yield is apparent for the samples which received hydrocarbon input. Humic substances were present in trace amounts only in the unaltered shale (samples 2 and 15). Interstitial gas (predominantly CH₄) from site 368 has been analysed for $\delta^{13}\text{C}$ content and composition¹⁷. The CH₄ becomes isotopically heavier with depth and is –51‰ between the sills. The yield of C₁ to C₄ hydrocarbons increases in the sill proximity. Both these results reflect the effect of the thermal stress from the intrusion.

Kerogen

The kerogen from unaltered Cretaceous black shale of site 41–368 was found to be immature¹¹. The results of the kerogen analyses are shown in Table 1 and data for samples 18 and 19 are quoted from Deroo *et al.*¹¹. The ash contents of the isolated kerogens were rather variable, and thus only the H/C and N/C values are calculated from the elemental analyses. A plot of H/C against depth in the sediment is shown in Fig. 2. A dramatic decrease of H/C : depth in the sill proximity is evident, indicating the aromatisation of the kerogen as a result of the thermal stress. The N/C values also exhibit a minimum in the sill proximity, however, the data are somewhat more scattered. A correlation plot of N/C against H/C is shown in Fig. 3. For comparison, additional data points are plotted for humic acids and kerogens isolated from diverse Recent environments, for example, lacustrine and marine sediments, peats, algal mats and soil¹⁸. The unaltered kerogens from site 41–368 cluster in the lower right portion of the plot and the altered kerogens cluster in

Fig. 2 Plots of the total hydrocarbons and the atomic H/C of the kerogens versus depth in the sediment. The intrusions are indicated by the hatched areas and the relative distance above and below the major sill is also given.

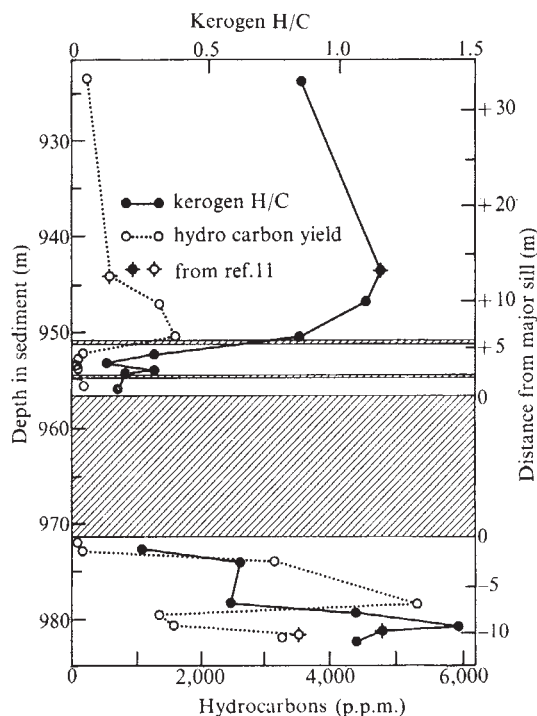


Table 1 Organic geochemical results for the samples from DSDP site 41-368

No.	Sample designation	Depth below seabed (m)*	Carbon (%)		CaCO ₃ (%)	Yield hydrocarbon† (μg g ⁻¹)	Pr/Ph	H/C	Kerogen		R ₀ (mode %)	
			Total	Organic					δ ¹³ C ³ (‰)	δD‡ (‰)		
1	58-2 (106-114)	923.6 [+32.9]	1.00	0.66	3	220	0.52	0.86	0.030	-25.01	-147	0.55
2	59-3 (composite)	~947 (942)	11.71	11.61	0.9	1,350	0.62	1.12	0.032	-26.43	-139	0.25
3	60-1 (121-127)	950.7 [+5.8]	7.62	3.03	38.2	1,600	1.24	1.12	0.100	-24.62	-70	1.05
4	60-2 (133-140)	952.4 [+4.1]	1.60	1.52	0.7	150	NR	0.316	0.025	-23.87	ND	1.45
5	60-3 (48-52)	953.0 [+3.5]	0.39	0.33	0.5	75	1.29	0.126	0.020	-24.50	+13	2.25
6	60-3 (122-126)	953.7 [+2.8]	6.81	6.78	0.3	60	2.86	0.319	0.026	-24.27	-95	2.35
7	60-4 (37-39, 48-51)	954.4 [+2.1]	2.38	2.36	0.2	50	1.86	0.209	0.027	ND	ND	2.65
8	60-5 (12-18)	955.7 [+0.8]	8.64	8.11	4.5	180	1.05	0.174	0.007	-23.87	-94	1.55
9	62-3 (28-34)	971.8 [-0.25]	0.32	0.07	2	70	0.78	Insufficient		-24.71	-72	—
10	62-3 (109-114)	972.6 [-1.05]	1.96	1.93	0.3	172	0.73	0.268	0.014	-26.62	-119	2.24
11	62-4 (106-110)	974.1 [-2.55]	6.74	6.70	0.3	3,110	1.12	0.633	0.052	-27.45	ND	0.95
12	63-1 (48-53)	978.5 (975.5) [-6.95]	4.77	4.24	4.4	5,300	1.02	0.61	0.029	-27.16	-123	0.45
13	63-2 (14-19)	979.7 (976.7) [-8.15]	3.70	3.59	1.0	1,350	0.92	1.05	0.038	-27.75	ND	0.45
14	63-2 (127-132)	980.8 (977.8) [-9.25]	6.64	6.56	0.7	1,540	0.80	1.43	0.037	-28.40	-115	0.35
15	63-3 (110-114)	982.1 (979.1) [-10.55]	8.56	7.10	12	3,250	0.55	1.06	0.026	-27.44	-147	0.35
16‡	58/59-2/3/4/3 (composite)	921 to 948 [+9 to +35]	2.70	2.33	3	680	0.68	ND	ND	ND	ND	ND
17‡	63-3/4 (composite)	971 to 984.5 (-9 to -13]	8.09	7.31	7	2,600	0.52	ND	ND	ND	ND	ND
18§	59-1 (97-107)	944.0 (939.5) [+12.5]	3.78	3.30	4	520	0.60	1.16	0.027	ND	ND	ND
19§	63-3 (17-27)	981.2 (978.2) [-9.65]	8.80	7.60	10	3,500	0.87	1.17	0.031	ND	ND	ND

ND, not determined; NR, not resolvable.

*Depths are calculated upwards from the core catcher, these are probably more representative of the true (absolute) depths than the DSDP convention of measuring downwards from the top of each core (if different, the DSDP is given in parentheses); the depths in brackets are the relative distances away from the major sill.

†Weight represents total hydrocarbon fraction (from thin layer chromatogram).

‡Values expressed versus standard PDB and SMOW for C and H, respectively.

§Data from ref. 11.

the lower left portion (that is, highly aromatic). The $\delta^{13}\text{C}$ and δD values both become heavier in the sill proximity and are $\geq -25\text{‰}$ for C and $> -100\text{‰}$ for H, whereas for the unaltered kerogens the values are $\leq -27\text{‰}$ for C and -140‰ for H (Table 1). This indicates a preferential loss of the lighter isotopes ^{13}C and H during the thermal activity. Similar effects for carbon isotopes have been observed by other workers^{19,20}.

Oil immersion vitrinite reflectance values for samples of each kerogen embedded in polished epoxy briquettes²¹ are presented in Table 1. The reflectance values (R_0) show correlations with the atomic H/C (correlation coefficient $r=0.86$) and $\delta^{13}\text{C}$ ($r=0.62$). The existence of these correlations between samples along such a steep thermal gradient tends to suggest that the dominant factor affecting these sediments is the Miocene thermal event rather than differences in the quality of the contributing organic matter. Maturation of kerogen results in increasing values of reflectance for the vitrinitic material, a preferential loss of hydrogen relative carbon, and a systematic increase in the $\delta^{13}\text{C}$ of the remaining kerogen.

The kerogen of sample 8 (Table 1) contains numerous vesiculated fragments of probable "coked" bitumen (N. H. Bostick, personal communication) composed of anisotropic domains or mosaic structure^{22,23} with characteristically high R_0 values. Smaller quantities of this material are also found in samples 6 and 7 further above the major sill (Fig. 1). The extremely low N/C ratio of sample 8 kerogen (Table 1) is within the range of most solidified bitumens²³. The vesi-

culation, mosaic structure and predominance of "coked" material between sills (Fig. 1) suggests that the extreme thermal alteration at this locality produced a liquid product from the organic matter which was unable to escape and apparently congealed to form solidified kerogenous bitumen.

The anomalously low R_0 value for vitrinite in sample 8 may be the result of the extremely high temperature imposed on the sapropelic type of organic matter which predominates in these sediments. For example, it has been shown that vitrinites from sapropel coals and shales have lower reflectance values than vitrinites of adjacent humus coals or shales²⁴. Extreme thermal stress on sapropelic material may result in the absorption of generated bitumen into associated vitrinitic kerogen. This may cause a retarded rate of reflectance maturation in the vitrinite.

Thus, the thermal effects of the basalt intrusions on the organic matter of the Cretaceous black shale from site 41-368 are discerned best in the following parameters: total hydrocarbon yield, kerogen H/C, kerogen $\delta^{13}\text{C}$, the presence of elemental sulphur, and vitrinite reflectance data (Table 1).

The hydrocarbons of the unaltered shale exhibit a biogenic distribution pattern of mixed autochthonous and terrigenous allochthonous origin. The hydrocarbons of the thermally altered samples exhibit a very narrow distribution pattern. A major and minor concentration of expelled volatile organic matter was detected ~7 m below and above the major sill, respectively. It seems that some of the volatilised organic matter migrated away laterally along

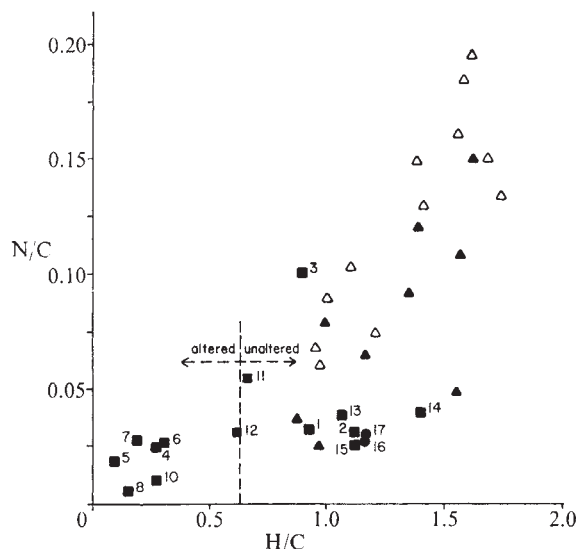


Fig. 3 Correlation plot of atomic N/C versus H/C for the kerogens. ■, kerogen; ●, kerogen, unaltered (ref. 11); ▲, kerogen (ref. 18); △, humic acid (ref. 18).

sand or carbonate layers above the sills.

The kerogen seems to become more aromatised (that is, hydrogen deficient—low H/C) in the sill proximity (Fig. 2), whereas the kerogens from the thermally unaltered sediment (samples 1, 2, 13–15) exhibit a more aliphatic character (high H/C). The $\delta^{13}\text{C}$ and D values of thermally altered kerogens are heavier than those of the unaltered

samples, indicating that the lighter isotopes ^{12}C and H were preferentially lost during the thermal events.

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Mapping of the residues responsible for the negative cooperativity of the receptor-binding region of insulin

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Insulin binding to its receptor leads to negatively cooperative interactions among the receptor sites. Studies with 29 insulin analogues (animal insulins and proinsulin, insulin-like growth factor and chemically modified insulins) which vary 1,000-fold in their affinity for the receptor and in their biological potency, suggest that a discrete invariable region on the surface of the insulin monomer is responsible for inducing the negative cooperativity. This domain comprises some of the eight carboxy-terminal residues of the B-chain and the A₂₁ asparagine. Burying of this 'cooperative site' in the dimerisation of insulin leads to a loss of negative cooperativity. A revised mapping of the insulin molecule is proposed, featuring distinct bioactive and cooperative sites.

INSULIN elicits its biological effects by binding to specific receptors located at the surface of target cells¹. Studies of the biological activities, receptor affinities and sequences of insulin from different animals strongly suggested that a largely invariable region on the surface of the insulin monomer may be the receptor-binding region^{2–8}. This region includes both A-chain residues (A₁ Gly, A₅ Gln, A₁₉ Tyr and A₂₁ Asn) and adjacent B-chain residues (B₂₄ Phe, B₂₅ Phe, B₂₆ Tyr, B₁₂ Val and B₁₆ Tyr). The involvement of these residues was also tested by studying the biological properties and receptor

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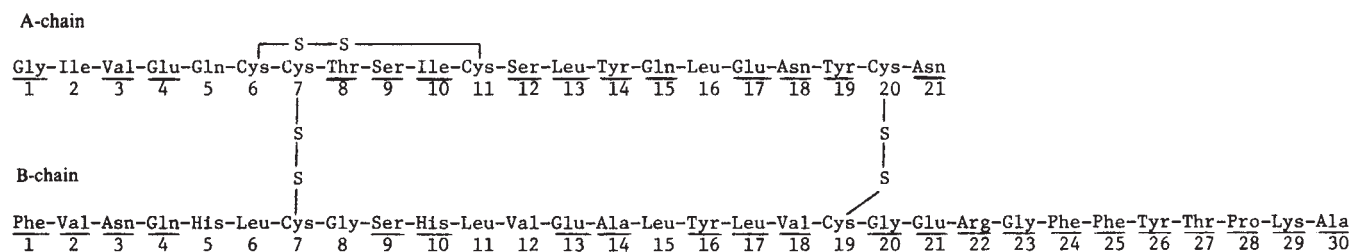


Fig. 1 The amino acid sequence of porcine insulin.

affinities of specifically modified insulins^{6,7}. The nature of the receptor binding site was recently further clarified by parallel studies of high resolution X-ray diffraction, circular dichroism, receptor binding and biological potency in fat cells⁸.

For a hormone to act on a target cell, it must not only bind to a receptor, but the combination of the hormone with the receptor must also activate the cell. The residues involved in receptor binding are not necessarily the same ones involved in triggering the biological effects of the hormone. However, for insulin, all analogues studied so far, although they vary widely in binding affinity, have shown parallel alterations in both binding affinity and biological potency and have behaved as full agonists possessing the same intrinsic activity. A possible exception was hagfish insulin, reported to have 5% biological potency relative to porcine insulin in the fat cell assay, but 25% relative binding affinity⁹; this higher binding affinity, however,

was not found by others in the lymphocyte (5%) or other receptors, including fat¹⁰. Thus, no chemical alteration has, so far, disclosed any discrete region of the structure that would be separately responsible for binding to the receptor and for activating the cellular processes once the hormone-receptor complex has been formed⁶. This is in contrast to other hormones (like ACTH) and pharmacological agents, where separate parts of the molecule must be implicated in receptor-binding and biological activation of the target cell, for some analogues behave as antagonists or partial agonists at the receptor level.

We report here results of our studies on the correlation between the ability of a variety of insulin analogues to bind to the insulin receptor and to elicit another consequence of receptor binding, a property that we have termed the 'negative cooperativity'^{11,12}. Negative cooperativity means that insulin binding to its receptor sites induces a loss of affinity of the other

Table 1 Insulin analogues studied

Analogue	Ref.	Gift from
Insulin analogues with intact cooperative properties		
Animal insulins		
Porcine	5, 19	R. E. Chance
Bonito fish	5, 20	R. S. Yalow
Mouse	5, 20	J. Schlichtkrull
Guinea pig	5, 20	L. F. Smith
Chicken	20	J. Simon
Hagfish	21	S. O. Emdin
Proinsulin derivatives		
Porcine proinsulin	22	R. E. Chance
Split proinsulin	23	R. E. Chance
Desdiptide	23	R. E. Chance
Diarginine	23	R. E. Chance
Insulin derivatives		
A ₁ 'thiazolidine' (pork)	4	T. L. Blundell
A ₁ 'thiazolidine' (beef)	4	T. L. Blundell
A ₁ acetoacetyl (beef)	24	T. L. Blundell
B ₁ acetoacetyl	24	T. L. Blundell
Des-Phe B ₁	25	D. Brandenburg
Des-Phe B ₁ Des-Gly A ₁	26, 27	D. Brandenburg
Desalanine	28	R. E. Chance
Monodesamido	28	R. E. Chance
A ₁ , B ₂₉ diaminosuberoyl	—	D. Brandenburg & Schermutski, unpublished
A ₁ , B ₂₉ diacetyl	29	D. Brandenburg
Carbonyl bis methionyl	30	W. Busse and F. H. Carpenter
Phenylene bis-thio carbamoyl-dimer (PBC-dimer)	31	D. Brandenburg
Tetranitro	32	F. H. Carpenter
Growth peptide		
NSILA-S (IGF)	33	R. Froesch
Insulin analogues with impaired or null cooperativity		
Despentapeptide (pork)	34	D. Brandenburg
Despenta/Deshexapeptide (human)	—	R. E. Chance (personal communication)
Desoctapeptide (beef)	28	F. H. Carpenter
Desalanine-Desasparagine (beef)	28	F. H. Carpenter
Maleyl (beef)	35	S. Wilson

receptor sites for insulin, due, in this case, to an accelerated dissociation rate of the insulin-receptor complex. Negative cooperativity seems to be a very fundamental property of the insulin receptor, as we have found it to be present for the insulin receptor of all animal species, including the receptor of the guinea pig, whose insulin has itself undergone marked deviation from the mammalian insulin, and of the Atlantic hagfish (*Myxine glutinosa*), the most primitive vertebrate known^{10,13}.

We investigated whether the whole receptor-binding region of insulin was involved in triggering the site-site interactions among the receptors, or whether there may be a discrete site or some subspecialisation within the receptor-binding region, as had been reported in other cooperative ligand binding¹⁴. We demonstrate that, in fact, a well defined domain within the receptor-binding region is responsible for inducing negative cooperativity. For this, we carried out parallel studies of receptor binding of several insulin analogues (native animal insulins, as well as chemically modified ones) and of their ability to accelerate dissociation of ¹²⁵I-insulin in the kinetic assay measuring negative cooperativity¹¹. Both binding and

negative cooperativity were studied in the human cultured lymphocyte assay^{11,15,16}.

Comparison of binding of insulin analogues and induction of negative cooperativity

The techniques for measuring insulin binding and negative cooperativity are described in detail elsewhere^{11,15-17} and briefly in the legend to Fig. 2. Our results in the lymphocyte system concerning the binding affinity of the analogues are in excellent agreement with previously published results in rat liver⁶, fat cells⁷ and lymphocytes¹⁸, and support the general hypothesis of Blundell *et al.* on the nature of the receptor-binding region of insulin²⁻⁸.

Table 1 lists the analogues studied, and the references where their specific modifications can be found. The residues modified in at least one of the analogues studied are underlined in the sequence of porcine insulin represented in Fig. 1. The analogues vary over nearly 1,000-fold in their relative affinity for the receptor (from chicken insulin, three times as potent as porcine insulin, to NSILA-S, 1/300th as potent).

The only derivatives for which receptor binding data had not been reported previously are tetranitroinsulin (20% affinity relative to porcine insulin), human despenta/deshexapeptide (20%), maleyl insulin (10%), and carbonyl bis methionyl insulin (4%). Our result with the despenta-peptide (22-25%) is close to those of Gliemann *et al.* (15-18%) (ref. 7), whereas the Chinese group reported almost full biological activity for this analogue³⁶. In terms of primary structure, we have thus studied modifications of 70% of the insulin sequence plus the whole connecting peptide of proinsulin.

Cooperative and non-cooperative analogues

The majority of the insulin analogues (Table 1) showed a perfect correlation between their relative abilities to occupy the insulin receptor and their potency in inducing the acceleration in the dissociation rate of insulin. We have termed these 'fully cooperative' analogues. Binding curves and cooperativity curves for some of these are shown in Fig. 2a. When the analogue/insulin concentration ratio for occupancy is plotted as a function of the ratio for negative cooperativity, most analogues fall along a line of slope 1, indicating perfect correlation between the two properties studied (Fig. 3). In particular, all the animal insulins, including the hagfish and guinea-pig insulins, show intact negative cooperativity, indicating that the residues important for negative cooperativity are mostly among the invariable ones.

In contrast, a few insulin derivatives showed a marked departure from this behaviour, as shown in Table 1 and Fig. 2b. Desoctapeptide (DOP) insulin is a derivative that has lost the eight carboxy-terminal amino acids of the B-chain. It has a reduced affinity for the receptor and a modified conformation similar to that of guinea-pig insulin³. DOP insulin is, however, capable, at high concentration, of saturating the insulin receptor sites like guinea-pig insulin. In contrast with guinea-pig insulin, this derivative is unable, even at concentrations 100-fold higher than the saturating ones, to induce the acceleration in the dissociation rate. The only other derivative showing a total loss of negative cooperativity is desalanine-desasparagine (DAA) insulin, lacking the last carboxy-terminal residue of both A-chain and B-chain. As desalanine insulin is fully active in both the binding assay and the assay for negative cooperativity, it must be the deletion of the A₂₁ asparagine that determines the loss of cooperativity of DAA. Desamidation of A₂₁, however, does not alter the cooperativity. Consistent with the loss of negative cooperativity of DAA and DOP insulins are the steeper competition curves for inhibition of ¹²⁵I-insulin binding by these two insulins, with 80% of the inhibition occurring within less than 2 log units of concentration, a characteristic of non-cooperative or 'statistical' binding (P.D.M., in preparation).

Despenta-peptide insulin (DPP), with only the last five carboxy-terminal B-chain residues missing, has an impaired

Fig. 2 Comparison between the ability of insulin analogues to inhibit ¹²⁵I-insulin binding to human cultured lymphocytes and their ability to induce negative cooperativity. *a*, Example of fully cooperative analogues; *b*, example of analogues with impaired or absent cooperativity. *Left*, binding assay. Cultured human lymphocytes are incubated in assay buffer at 15 °C for 90 min with a tracer amount of ¹²⁵I-insulin alone or in the presence of increasing concentrations of unlabelled analogue. The inhibition of the binding of ¹²⁵I-insulin by the various concentrations of analogues is plotted in % of the maximal inhibition obtained, representing the extent of specific binding (usually 95% of the initial binding) as a function of analogue concentration. (For precise assay conditions, see refs 16, 17.) ●, Insulin; ■, fish insulin; ▲, split proinsulin; ×, proinsulin. Kinetic assay for negative cooperativity (right). The assay is based on the property of cooperative analogues to accelerate the dissociation rate of a tracer amount of ¹²⁵I-insulin bound to the receptors. After binding of the ¹²⁵I-insulin to the lymphocytes has reached a steady state, an aliquot of the ¹²⁵I-insulin receptor complex is diluted 100-fold in the absence or presence of increasing concentrations of unlabelled analogue in the diluting medium. The dissociation is allowed to proceed for 30 min, after which the tubes are centrifuged and the amount of radioactivity still bound is measured. The data are plotted as the radioactivity dissociated in the presence of a given concentration of the analogue, minus the dissociation in the absence of the analogue, in % of the maximal effect, as a function of the analogue concentration. (For precise assay conditions, see refs 11, 15 and 17.) ●, Insulin; ■, DAA; ○, DPP; △, DPP/DHP; ▲, DOP.

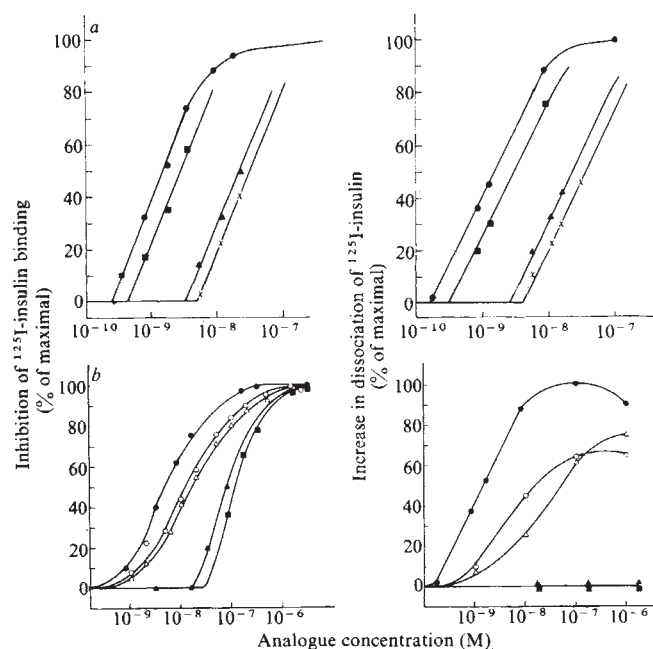
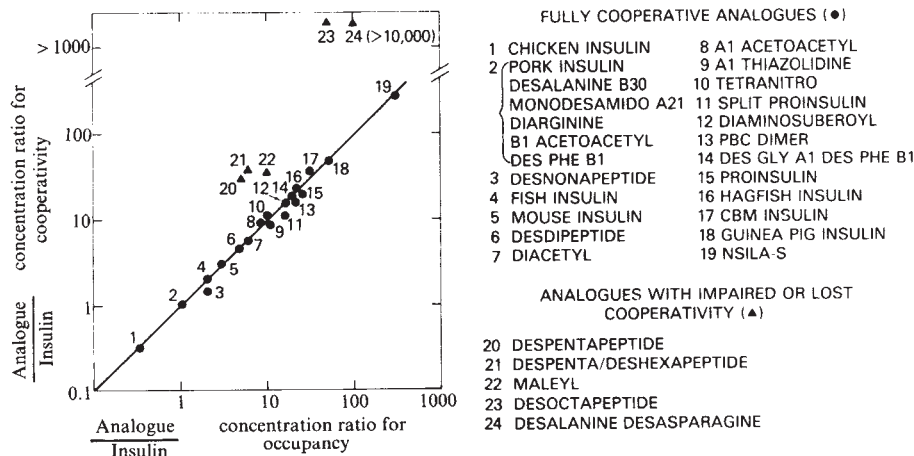


Fig. 3 Reciprocal potency ratios for receptor occupancy and negative cooperativity. The ratio of the concentration of an analogue required to obtain a 50% inhibition of insulin binding to the concentration of insulin required to obtain the same inhibition (a measure of their relative abilities to occupy the insulin receptor) is plotted as a function of the ratio of concentrations required to obtain 50% of the maximal acceleration of the dissociation rate (a measure of the relative abilities to induce negative cooperativity). The numbers refer to the table on the right. The fully cooperative analogues all fall on a straight line of slope 1, whereas analogues with impaired cooperativity will be above the line. Two analogues (DAA and DOP) where no cooperativity at all could be measured are represented above the break in the scale for cooperativity at their correct ratio for occupancy.



capacity to induce negative cooperativity (about 15% of the effect expected from the occupancy ratios). A mixture of 40% despentapeptide (five residues missing) and 60% deshexapeptide insulin (six residues missing) shows a further impaired negative cooperativity (10% of that expected). Thus, progressive deletion of from five to eight of the carboxy-terminal residues of the B-chain leads to a progressively complete loss of negative cooperativity. We were, unfortunately, unable to test distri- and destetrapeptide insulins. The B₂₉ Lys is probably not involved, as it is altered in several fully cooperative analogues. Maleyl insulin, acylated on B₁ and B₂₉ and partially on A₁, also has an impaired negative cooperativity; because other alterations at A₁, B₁ and B₂₉ are without effect, the loss of cooperativity in this case may be due to the presence of a negative charge at B₂₉, which may interfere with the binding of the neighbouring B-chain residues.

A 'cooperative site' on the insulin surface

It must be remembered, in interpreting these results, that the observed effects may be due not only to the absence or modification of an important residue or to the direct interference of a modifying group, but also to a change in the overall conformation of the insulin molecule. Hence, it may be that DAA and DOP insulins have lost the ability to induce negative cooperativity, not because of the loss of their terminal residues, but because of their highly altered conformation in solution. We do not think that this is so because negative cooperativity is absent at concentrations at which these derivatives have maximal biological activity and saturate all the receptor sites. Hence, by increasing their concentration, we probably obtain a sufficient fraction of conformers in the conformation required for receptor binding and biological activation, consistent with the full intrinsic activity of these derivatives. Also, some other derivatives with a tertiary conformation altered to an extent comparable to DAA and DOP insulin, for example, guinea-pig insulin³ or Des-Gly Des-Phe insulin, exert full cooperativity.

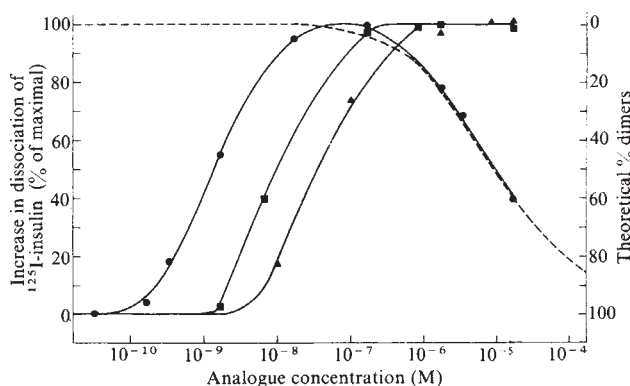
It is interesting that all the modifications that lead to a loss of cooperativity are located at the A₂₁ residue or at the eight carboxy-terminal residues of the B-chain, which come very close together at the surface of the insulin monomer (with a salt bridge between A₂₁ asparagine and B₂₂ arginine). This surface area, which constitutes a hydrophobic core within the putative receptor binding site of insulin²⁻⁸, may then represent an individualised 'cooperative site' at the surface of insulin specifically responsible for inducing negative cooperativity among the receptor sites. Modifications of the residues in the region of A₁ crucial for receptor binding and biological effects are not important for the induction of negative cooperativity. The individualisation of such a cooperative sub-site has similarly been reported in NAD binding to glyceraldehyde-phosphate dehydrogenase¹⁴, where negative cooperativity is triggered at the adenine sub-site.

The effect of insulin dimerisation

Because the putative cooperative site outlined above is also involved in inter-monomer contacts in the dimer of insulin^{2,4}, negative cooperativity should be affected by the dimerisation process. Indeed, as we have reported^{11,15}, the negative cooperative effect falls as the insulin concentration in the medium is increased above 10⁻⁷ M. Strikingly (Fig. 4), this fall follows perfectly the theoretical curve for dimerisation of insulin at neutral pH (ref. 37). In contrast, two insulin analogues that do not dimerise at high concentrations, guinea-pig insulin^{38,39} and tetranitrotyrosyl insulin³², show no tendency to a decrease in negative cooperativity at very high concentration (Fig. 4). This supports the idea that the area comprising the A₂₁ asparagine and the carboxy-terminal residues of the B-chain are indeed those involved in negative cooperativity. This disappearance of cooperativity as a function of dimer formation also implies that the dimers bind to the receptor, for if dimers did not compete for monomer binding, the absolute concentration of monomers at these high concentrations would still be sufficient to saturate the sites and elicit negative cooperativity.

These findings also eliminate the possibility that negative cooperativity is due to insulin dimer formation, as suggested by Cuatrecasas *et al.*⁴⁰. Indeed, negative cooperativity is induced by non-dimerising insulins and, on close inspection, is unrelated to the capacity of insulin analogues to form dimers (Table 2).

Fig. 4 Effect of insulin dimerisation on negative cooperativity. The ability of pork insulin and two non-dimerising insulins to induce negative cooperativity are measured in the same kinetic assay as Fig. 2, right. The assay includes high concentrations of the analogues, where dimerisation of porcine insulin becomes significant. The dashed line (note the inverted scale) represents the theoretical curve for dimerisation [mols dimer/mols (monomer + dimer)] × 100 calculated from a $K_{dimer} = 2.22 \times 10^5$ at neutral pH (ref. 37). ●, Pork insulin; ■, tetranitroinsulin; ▲, guinea-pig insulin.



Conclusion

We have presented data supporting the hypothesis that a specialised region of the insulin monomer surface is responsible for regulating the affinity of the insulin receptor (negative cooperativity). Thus, both insulin and its receptor must contain a specialised domain, a 'cooperative site', the binding of which results in a regulatory decrease in the affinity of the complex. A surface mapping, based on that presented by Blundell *et al.*, but including our cooperative site, is presented in Fig. 5.

It is worth noticing that negative cooperativity is the only property of insulin that has been as tightly conserved through evolution as the intrinsic biological activity of insulin itself, and is apparently invariable throughout the entire history of vertebrate evolution, extending nearly a half billion years. Negative cooperativity is present in both the insulin molecule and the receptor even in species where other properties are altered, such as affinity for binding (non-mammalian and hysticomorph insulins), the ability to dimerise (guinea pig insulin), to bind zinc (guinea pig and hagfish insulins), and possibly even the intrinsic activity (hagfish insulin). Even non-suppressible insulin-like activity (NSILA-S), a mixture of at least two insulin-like growth-promoting peptides with a weak affinity (1/300 porcine insulin) for the insulin receptor, has full ability to induce negative cooperativity. Interestingly, the recently disclosed partial sequence³³ of IGF-I includes the 'cooperative' B-chain residues, B₂₃ Gly, B₂₄ Phe, B₂₅ Phe and B₂₆ Tyr, which are nearly identical to insulin except for the B₂₅-B₂₆/Phe-Tyr inversion.

It should be emphasised that the cooperative site outlined above probably contributes substantially to the free energy of binding insulin to the receptor. Indeed, it comprises most of the non-polar invariable residues of the putative insulin binding

Table 2 Comparison of dimerisation and negative cooperativity activity of different insulins

Insulin	Dimerises at high concentration	Cooperativity	
		At low concentration	At high concentration
Pork insulin	Yes	Yes	No
DAA insulin	Yes	No	No
Hagfish insulin	Yes	Yes	Yes
Guinea-pig insulin	No	Yes	Yes
Tetranitroinsulin	No	Yes	Yes
DOP insulin	No	No	No

Hagfish insulin, although able to form dimers, conserves its ability to induce negative cooperativity at high concentrations; however, there are significant differences between the hagfish dimer and the porcine dimer in regions involving both ends of the B-chain⁴¹.

surface, and recent thermodynamic studies (ref. 46 and M. Waelbroeck, E.V.O. and P.D.M., in preparation) support the hypothesis of Blundell *et al.* that burying these non-polar residues in the hormone-receptor complex (hydrophobic effect) is a major driving force in the reaction of insulin with the receptor.

Our conclusions will clearly require further substantiation through parallel studies of X-ray analysis, dimerisation and receptor binding of more analogues. It should be stressed that our findings do not depend on the precise molecular nature of negative cooperativity, a concept with multiple possible physical interpretations. Whatever its basis, the phenomenon of accelerated dissociation has added a further dimension to the studies of the structure-activity relationship of insulin. This also has potential implications in drug design, making theoretically possible the synthesis of an insulin with intact affinity that may stay longer on its receptor.

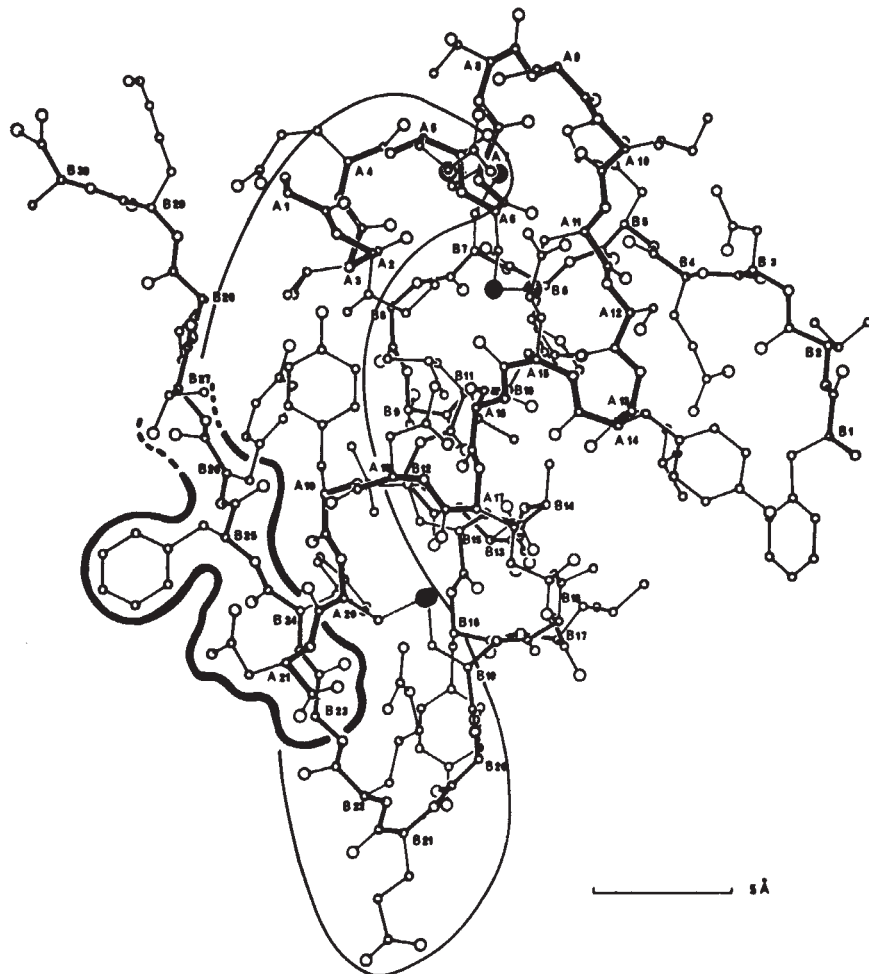


Fig. 5 Mapping of the cooperative site on the insulin monomer. The solid line represents the putative receptor-binding region ('bioactive site') (after ref. 3); the thick line the cooperative site. (Figure due to the courtesy of T. L. Blundell.)

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Calcium transients in aequorin-injected frog cardiac muscle

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The Ca^{2+} -sensitive bioluminescent protein aequorin was microinjected into cells of frog atrial trabeculae to study intracellular calcium transients associated with excitation-contraction coupling. The amplitude of the aequorin signal increased with extracellular Ca^{2+} concentration and stimulus frequency, but decreased with stretch. Isoprenaline and acetylcholine both increased the amplitude, but had strikingly different effects on the time course of the signal.

A CHARACTERISTIC property of cardiac muscle is that the tension developed or shortening accomplished in a contraction can vary over a wide range even though all cells are excited in every contraction. Variations in contractility can be produced by numerous means, such as changes in the frequency of contraction, alterations in the ionic composition of the bathing fluid, and the application of certain drugs. An increase in intracellular Ca^{2+} concentration is thought to be the event responsible for activating the contractile machinery¹ and it is widely supposed that alterations in contractility are caused by changes in this calcium transient². Although it has long been customary to draw a sharp distinction between inotropic effects of the sort listed above and changes in performance produced by changes in fibre length, there is increasing doubt that the mechanisms involved are entirely distinct³. Direct evidence concerning the role of changes in intracellular calcium transients in the regulation of myocardial performance would help to clarify this and many other uncertainties, but has had to await the

development of a suitable intracellular calcium indicator. The most promising such agent to appear so far has been the calcium-sensitive bioluminescent protein aequorin, extracted from the jellyfish *Aequorea forskåle*^{4,5}. Aequorin has been used as a calcium indicator inside a variety of cells⁶, including barnacle muscle⁷ and frog skeletal muscle^{8,9}, but attempts to apply it in the much smaller cells of heart muscle have not previously been successful. We report here the microinjection of aequorin into the cells of frog atrial trabeculae, and describe the results obtained from six experiments.

Thin (75–150 μm diameter) unbranched trabeculae were isolated from the atria of frogs (*Rana pipiens*). They were mounted in a bath of Ringer solution, with their ends impaled on two hooks, one of which was attached to a tension transducer. Five-millisecond pulses were applied to parallel platinum plates to stimulate the muscle. Light was detected with a photomultiplier and the resulting signal (time constant 10 ms) was stored on magnetic tape along with the tension and stimulus signals. Signals were averaged digitally to reduce noise in the light tracing. The apparatus and methods used were essentially as described for skeletal muscle⁹, except as noted below.

Microinjection of aequorin

Aequorin prepared as described elsewhere^{5,10} was dissolved to a concentration of about 4×10^{-5} M in 150 mM KCl, 1 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid) buffer, pH 8. Pipettes for microinjection were pulled from glass capillary tubing with a filament fused to the inside to facilitate filling the tips; when filled with aequorin solution, these had resistances of 100–200 M Ω . Gas pressures of up to 10 atm were applied to eject

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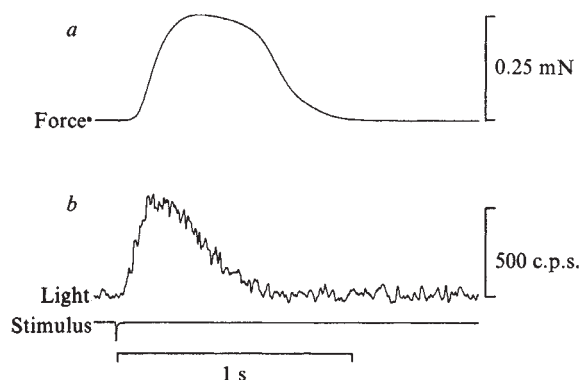


Fig. 1 Mechanical (a) and luminescent responses (b) from an aequorin-injected atrial trabecula of *R. pipiens*. Isometric contractions at 0.5 Hz; length about $1.3 \times L_0$; 21 °C (128 sweeps averaged). Ringer solution contained (mM) Na^+ 120.2, Ca^{2+} 1.8, K^+ 2.5, Cl^- 121.1, phosphate 3; pH 7.2. Light signals are calibrated in photon counts per second (c.p.s.).

aequorin. The patency of each pipette was established by recording the light produced when aequorin was ejected into the Ringer solution. Cells on the upper surface of the undamaged region of the trabecula were then penetrated, and pressure was applied for short periods. (Cells in this preparation are tapered, less than 10 μm in greatest diameter, and about 200 μm long¹¹; each trabecula contains several thousand cells.) The membrane potential was monitored as an indicator of penetration. An apparently satisfactory injection was considered to have occurred when a stable membrane potential more negative than -70 mV was obtained, and application of pressure for a few seconds resulted in little change in the potential. In a given preparation 30–90 cells were injected in this way before usable light signals were obtained. We do not know how many of these injections resulted in undamaged cells that contained aequorin and contributed to the overall light signal. The number of such cells was undoubtedly less than the number of apparently successful injections, because pipettes often became plugged and some of the apparently satisfactory injections probably did cause cell damage. To relate the amplitude of the recorded aequorin signals to the amount of aequorin actually present in the muscle, we terminated four experiments by recording the light emitted when all of the aequorin in the preparation was suddenly discharged. This was done by rapidly mixing the detergent Triton X-100 (final concentration approximately 0.5%) into the bathing solution without altering the geometrical relation between the muscle preparation and the photomultiplier. This lysed the cell membranes, causing all of the aequorin in the preparation to be discharged by Ca^{2+} within about 15 s.

Characteristics of the aequorin signal

After the period of injection, the light and tension responses under any given set of experimental conditions were generally stable for many hours and many thousands of contractions. This permitted signal averaging, which was necessary to obtain good signal-to-noise ratios for the luminescent responses in even our best-injected preparations. No light was detected from preparations at rest. Figure 1 shows the mechanical and luminescent responses to stimulation in a preparation with a relatively strong light signal. The light signal rose well before the tension and reached a peak 150–250 ms after the stimulus at 21 °C and 0.5 Hz stimulus frequency. As in other types of muscle so far investigated^{7–9} the peak of the luminescent response occurred at about the time of maximum rate of increase in tension. The decline of the aequorin signal was slower

than the rise, and luminescence became indistinguishable from the background noise about 600 ms after the stimulus. The responses shown in Fig. 1 look superficially similar to those observed in the twitches of aequorin-injected skeletal muscle fibres^{8,9}, but there are two important differences. First, the time course is much slower in cardiac muscle and the light response is thus relatively undistorted by the limited ability of the aequorin reaction to follow rapid changes in Ca^{2+} concentration¹². Second, for a given amount of aequorin injected, the peak light intensity during a contraction is very much lower (by a factor of about 50) in cardiac muscle than in skeletal muscle.

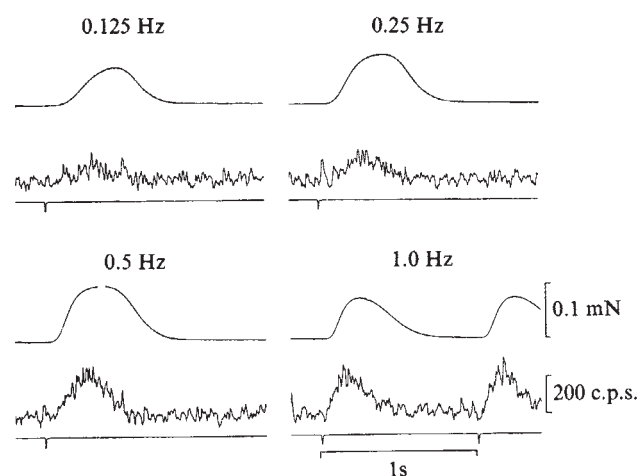
Effects of inotropic interventions

The influence of the frequency of contraction on tension and light output is shown in Fig. 2. In this experiment the light response was only barely detectable at the lowest frequency (0.125 Hz) used, but increased substantially as the frequency was increased up to 0.5 Hz. At higher frequencies both the light signals and the contractions were abbreviated.

Figure 3 illustrates the influence of extracellular Ca^{2+} concentration. Like changes in frequency, changes in Ca^{2+} concentration have more striking effects on the amplitude of the light signal than on the tension developed. Although the mechanical response is somewhat prolonged by increasing Ca^{2+} concentration, there is no obvious influence of Ca^{2+} concentration on the duration of the aequorin signal.

The catecholamines and the cardioactive steroids are the most important groups of drugs with inotropic actions. Figure 4 shows the effects of maximally inotropic concentrations of representatives of each of these groups—(-)-isoprenaline and acetylthioflavine, respectively. Although these drugs increased the force of contraction to about the same extent, they produced strikingly different changes in the luminescent responses. Isoprenaline increased the amplitude of the aequorin signal very much more than did acetylthioflavine. With isoprenaline, the light intensity increased in two phases—a rapid initial phase lasting about 25 ms, and a slower secondary phase (lasting about 200 ms) that continued almost to the time of peak tension. Light intensity then decreased very rapidly, approaching the baseline much more abruptly than in the absence of the drug (see Fig. 1). (This rapid decline was associated with the rapid relaxation characteristically produced by catecholamines.) With acetylthioflavine, on

Fig. 2 Influence of frequency of contraction on tension development and the aequorin signal. Length about $1.3 \times L_0$; 21 °C (128 sweeps averaged at each frequency).



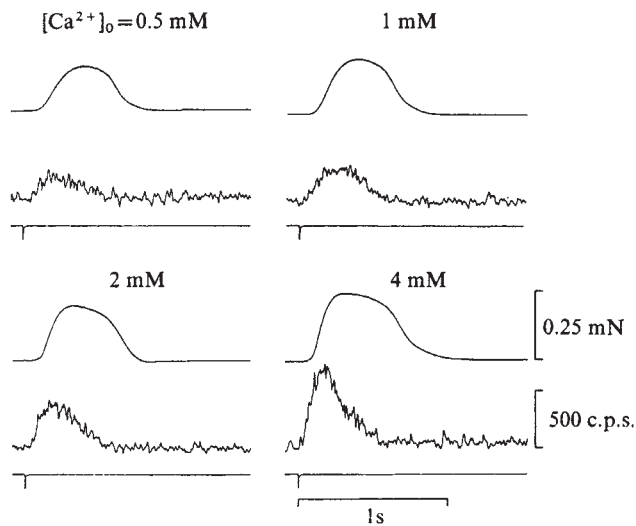


Fig. 3 Influence of extracellular Ca^{2+} concentration on tension development and the aequorin signal. Length about $1.3 \times L_0$; 0.5 Hz; 21°C (128 sweeps averaged).

the other hand, the aequorin response had only a rapidly rising phase. This was of about the same amplitude and duration as the rapid initial phase of the response in isoprenaline, but was followed immediately by a gradual decline of light intensity that lasted nearly a second. The luminescent response had reached its peak and was declining well before the rate of tension development had become maximal.

Changes in muscle length produced substantial changes in the amplitude of the aequorin signals, but in a direction opposite to that of the mechanical responses (Fig. 5). In these experiments we expressed measurements of muscle length in terms of L_0 , the length (between hooks) at which the muscle was just slack. Below this length the muscle assumes a curved shape and must shorten before tension can be developed; at lengths above L_0 the preparation as a whole was virtually isometric. In the experiment of Fig. 5 muscle length was varied between $0.8 L_0$ and $1.4 L_0$ (greater stretch tended to enlarge the holes where the hooks were inserted), and the amplitude of the aequorin signal decreased progressively over this range. Similar results were seen in two other preparations. Although we did not measure sarcomere lengths in any of the preparations injected with aequorin, we subsequently did estimate them from laser diffraction patterns in several trabeculae of similar size. At and below L_0 the sarcomere lengths were about $2.0 \mu\text{m}$ at rest; at lengths below L_0 there was a considerable decrease in sarcomere length during contraction. As the muscle was stretched above L_0 the sarcomere length increased, but proportionally somewhat less than muscle length. During contraction some parts of the preparation shortened, whereas others lengthened. We could rarely define unequivocally a length at which the developed tension was maximum (because of deformation of the muscle ends), but in all preparations the tension development was greater at a resting sarcomere length of $3.0 \mu\text{m}$ than at shorter lengths. This finding is in accord with that of Nassar *et al.*¹³, on the same type of preparation.

Quantitative aspects

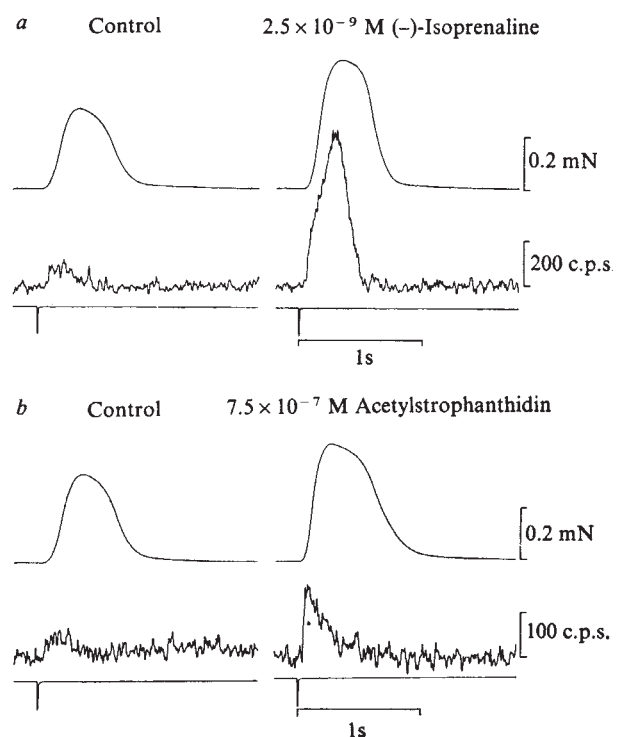
In Fig. 6 the light and force signals obtained during a Triton X contracture are shown together with those previously recorded during normal contractions of the same preparation. From these measurements alone it is possible to estimate the fraction of the injected aequorin consumed in a contraction. With additional information one can use the measurement of the total amount of light emitted during the Triton X contracture to estimate the amount

of aequorin injected, and also the intracellular Ca^{2+} concentration corresponding to any given light intensity measured during the experiment.

To estimate the fraction of the injected aequorin consumed in a normal contraction, we determined the ratio of the area (light intensity $\times$ time) under the light record made during a twitch to that under the record obtained during the terminal Triton X contracture. In four experiments the mean of this fraction was 3.5×10^{-5} (range 9.0×10^{-6} to 5.5×10^{-5}). Some additional aequorin was undoubtedly consumed while the preparation was at rest, but the virtual constancy of the light signals in preparations stimulated only occasionally over periods as long as 24 h indicates that the rate of consumption at rest must have been very low.

From the light emitted during Triton X contractures, a measurement of the light yield per unit volume of the aequorin solution injected, a correction for the optical geometry of the experimental set-up, and the estimated total aequorin consumption during the contractions of the experiment (6–24%), we have calculated that the average volume of aequorin solution injected must have been about 280 pl (range 230–340 pl in four experiments). From the number of apparently satisfactory injections, the average volume injected per cell is estimated to have been about 5 pl (range 2.5–11 pl in four experiments). If, as we have argued above, the number of truly successful injections was smaller than the apparent number, the volume per injection must have been correspondingly larger. The volume of the cells in the atrial trabeculae is probably no more than 5–10 pl (ref. 11), and we must assume that the final intracellular aequorin concentration was in the vicinity of half the concentration injected, or about $2 \times 10^{-9} \text{ M}$.

Fig. 4 Influence of isoprenaline (a) and acetylcholinesterase inhibitor (b) on tension development and the aequorin signal. Records from the same preparation; length about $1.3 \times L_0$; 0.5 Hz; 21°C . Note that sensitivity of light recordings in b is twice that in a (256 sweeps averaged in all records). Full dose-response curves for the drugs were determined on similar preparations that had not been injected with aequorin. The concentrations used here were chosen to be just maximally inotropic.



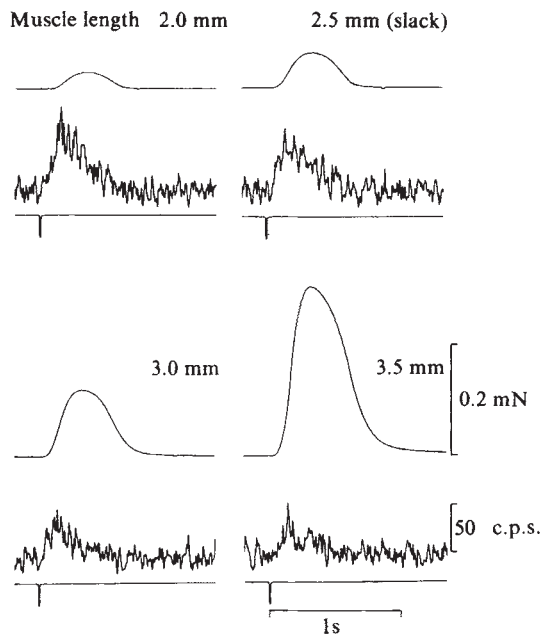


Fig. 5 Influence of muscle length on tension development and the aequorin signal. Stimulus frequency 0.33 Hz; 21 °C. The muscle was slack below 2.5 mm (256 sweeps averaged).

Our approach to calibrating the light signals during contraction in terms of Ca^{2+} concentration depends on relating the light intensity recorded during a contraction to an estimate of that which would have been recorded had all the aequorin in the preparation been exposed simultaneously to a saturating concentration of Ca^{2+} . The latter is estimated by multiplying the total amount of light emitted during the Triton X contracture by the rate constant of aequorin consumption in the presence of a saturating concentration of Ca^{2+} (1.0 s^{-1} at 21 °C.) (The process is shown diagrammatically in Fig. 6. The dashed line superimposed on the light signal recorded during exposure to Triton X subtends the same area as the recorded curve, but has the same time course as the light signal emitted when aequorin is rapidly mixed with a saturating concentration of Ca^{2+} at 21 °C *in vitro*.) By relating the light signals recorded during contractions to the estimated maximum (the amplitude of the dashed curve), it is possible to normalise those signals to the amount of aequorin present in the muscle. This approach depends on the assumptions (verified experimentally) that the total amount of light emitted by a given amount of aequorin is independent of the speed with which the aequorin is discharged and not influenced by the presence of Triton X-100.

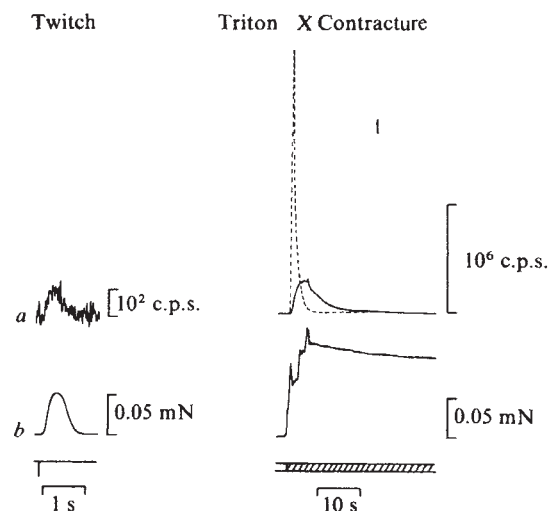
In four experiments the mean ratio of peak light intensity achieved during contractions at 0.5 Hz to the estimated maximum was 1.2×10^{-4} (range 3.6×10^{-5} to 2.5×10^{-4}). This ratio can be used in conjunction with an appropriate Ca^{2+} concentration-effect curve determined for aequorin *in vitro* to estimate the peak intracellular Ca^{2+} concentration achieved during contraction. For this purpose, the concentration-effect curve must have been determined over a wide range of Ca^{2+} concentrations in conditions appropriate to the intracellular environment; and light intensities should be expressed as fractions of the maximum obtainable in a saturating concentration of Ca^{2+} , as in the curve recently published by Allen *et al.*¹⁴. On the basis of the assumption that the intracellular Mg^{2+} concentration is close to 2 mM (refs 15 and 16), we have applied a calibration curve determined at 21 °C in 150 mM KCl, 2 mM Mg^{2+} and 5 mM PIPES (piperazine-*N,N'*-bis(2-ethanesulphonic acid)) buffer, pH 7. On this curve the ratios of light intensities

observed in the four experiments correspond to Ca^{2+} concentrations between 1.1 and $2.7 \times 10^{-6} \text{ M}$ (mean $2.0 \times 10^{-6} \text{ M}$). The possibility must be considered, however, that there are unknown aspects of the intracellular environment capable of influencing aequorin luminescence which we have not taken into account in determining our calibration curves.

Three other potential sources of error deserve mention. (1) Aequorin itself binds Ca^{2+} , and thus may lower the concentration it is intended to measure¹⁷. We estimate that during contraction about 10^{-6} M Ca^{2+} may be bound to the $2 \times 10^{-5} \text{ M}$ aequorin in the injected cells. This is about half the estimated free Ca^{2+} concentration in the myoplasm, so that at worst our estimate of Ca^{2+} concentration might be low to the extent of about one-third. In reality, the error is likely to be much less than that because of the presence of other calcium binding substances inside the cell. (2) One consequence of the non-linear relationship between Ca^{2+} concentration and light intensity¹⁴ is that when the distribution of Ca^{2+} is uneven, the light emission from regions of high Ca^{2+} concentration is disproportionately intense. Our estimate of intracellular Ca^{2+} concentration will be higher than the mean to the extent that gradients of Ca^{2+} concentration exist in the cell. (3) Though unlikely, it is conceivable that some cells were damaged by the injection in such a way that they did not generate calcium transients, yet maintained a Ca^{2+} concentration low enough to preserve the injected aequorin. If such cells were present, our estimate of the intracellular Ca^{2+} concentration during activity would have been too low.

It should be noted that because of the non-linearity of the Ca^{2+} concentration-effect curve for aequorin¹⁴, changes in light intensity tend to give an exaggerated impression of the changes in Ca^{2+} concentration responsible for them³. Thus, in Fig. 3, although the light signal increased by a factor of three when the extracellular Ca^{2+} concentration was increased from 0.5 to 4.0 mM, the peak intracellular Ca^{2+} concentration will have increased by a factor of only about $2.5\sqrt{3} = 1.55$.

Fig. 6 Method used to relate light intensity recorded in physiological conditions to that when all of the aequorin in the cell is suddenly discharged. Records on the left are of light (a) and force (b) recorded during twitches at 0.33 Hz (128 sweeps averaged). Records on the right were made in identical optical conditions (though with greatly reduced sensitivity for the light record) as 0.5% Triton X-100 was flushed into the bath (cross-hatched bar). Length about $1.3 \times L_0$; 21 °C. The detergent destroys cell membranes, allowing all of the aequorin in the cell to be discharged by Ca^{2+} . The dotted line encloses the same area as the recorded curve, and shows the time course of the flash that would have been obtained had the aequorin in the cell been exposed instantly to a saturating Ca^{2+} concentration.



Discussion

The results shown in Figs 1, 2 and 3 are at least qualitatively what one might have predicted on the basis of existing knowledge about excitation-contraction coupling in frog heart muscle¹⁸. The gradual upstroke of the calcium transient in basal conditions (for example, Fig. 1) is consistent with the concept that in frog heart most or all of the calcium involved in activation enters the cell from the extracellular space during the plateau of the action potential. The slow rate of rise of the Ca^{2+} concentration is presumably the basis for the observation¹⁹ that the active state seems to rise slowly in heart muscle (in contrast to frog skeletal muscle; see discussion in ref. 20). Our estimate of the Ca^{2+} concentration during activity in heart muscle is only about one-fifth of that for a tetanus in skeletal muscle⁹. This is consistent with the common supposition, based on differences in the contractile behaviour of the two types of muscle, that the calcium transients rise to a level sufficient to saturate the contractile apparatus in frog twitch fibres, but not in heart muscle.

The very rapid initial rise in light intensity in the presence of high concentrations of isoprenaline (Fig. 4a) and acetylthiocholine (Fig. 4b) suggests that in these conditions at least, the release of Ca^{2+} from intracellular stores may play a significant role in activation. This is consistent with the finding that substantial releasable Ca^{2+} stores are present in frog heart²¹.

The decrease in light output produced by increases in fibre length (Fig. 5) was unexpected and is difficult to reconcile with other information. It is not easy to see how it could have been an optical artefact of stretching the tissue. The aperture of the photomultiplier (25 mm) was very much larger than the preparation (< 5 mm); the preparation was checked under the microscope and observed not to rotate during stretch; three different preparations gave similar results; aequorin signals from skeletal muscle fibres also decrease with stretch over the same general range of sarcomere lengths^{8,9}.

There is increasing reason to believe that changes in activation as well as changes in filament overlap are involved in the operation of Starling's law of the heart². Although there is considerable variation in thin filament length in frog atrial trabeculae^{22,23}, the mean lengths of the thick and thin filaments are similar to those of frog skeletal muscle²², and on the basis of filament overlap alone, one would expect contractile tension to decrease with stretch over the range of sarcomere lengths covered in our experiments. However,

the situation is complicated by the variable amounts of shortening that occur during contractions. This shortening affects the interpretation in a number of ways: it means that the resting sarcomere spacings are not appropriate to the active muscle; it leads to 'mechanical deactivation' of tension development²⁴; and there is recent evidence from frog skeletal muscle that shortening can affect the intracellular Ca^{2+} concentration²⁵. Nevertheless, the fact that tension development increased while filament overlap and the amplitude of the calcium transient both decreased is puzzling. If no artefact can be identified to account for this discrepancy, it may be necessary to attribute the prime influence of stretch on cardiac contractility to a change in the sensitivity of the contractile apparatus to Ca^{2+} , such as has been described by Endo in skinned skeletal muscle fibres²⁶, and by the Fabiato in cardiac muscle²⁷.

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Characteristics of successful natural enemies in models of biological control of insect pests

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Biological control of insect pests is characterised by a persistent, strong reduction in the pest population following the introduction of a natural enemy. Analysis of mathematical models suggests that differential exploitation of patches of the pest in a spatially heterogeneous environment provides the most likely mechanism to account for known successes.

BIOLOGICAL control of insect pests by means of natural enemies, usually imported insect parasitoids (Hymenoptera and Diptera) or, more rarely, predators, has achieved considerable success¹. Surprisingly, the reasons for the success of some programmes and the failure of others are very poorly understood. Here we attempt to identify those features of the interaction between an insect pest and a natural enemy that permit successful biological control.

Most of the study is concerned with insect parasitoids because mathematical models of host-parasitoid interactions are both easier to construct and more realistic than those for predators and their prey, and involve fewer parameters, to which biologically sensible values can be assigned with reasonable confidence. Field and laboratory data against which predictions can be tested are also more easily obtained for parasitoids. In this article we first measure the impact of natural enemies on several field and laboratory populations and then attempt to reproduce these effects in mathematical models of host-parasitoid interactions. Our criterion for realism in these models is that they must permit the coexistence of the two populations, whilst accurately reflecting the characteristic levels of abundance of the host in both the presence and absence of the parasitoid. The mathematical techniques used in the analysis of the models have been reported elsewhere².

Field and laboratory data

Formally, we can measure the impact that a parasitoid has on its host by a simple ratio—the equilibrium population size of the host in the presence, N^* , and absence, K , of the natural enemy. We call this ratio q . Figure 1 shows q values estimated from several successful biological control programmes and from four laboratory systems. Estimates were made by averaging a series of counts of the host density first in the absence and then in the presence of the parasitoid. Such estimates are inadequate for careful comparison with detailed predictions, but we are here concerned only with order-of-magnitude effects and the data are quite adequate for this purpose.

The laboratory populations all involve only a single species of host and its parasitoid. Similarly, the field examples chosen are interactions in which a single species of natural enemy is credited with the successful control of a former pest. Note that the depression (q) of the field populations is much more marked than the depression of the laboratory populations. This is not due to a conscious selection of data; the results shown in Fig. 1 represent all the studies known to us for which even crude estimates of q can be made. Reviews of biological control work suggest that dramatic depression of the host population in the field (very low q) is common, but by no means inevitable^{1,3,4}. We now compare these field and laboratory values for q with the minimum values that can be generated from biologically feasible mathematical models of host-parasitoid interactions.

Host depression in simple population models

In previous work^{2,5} we explored q values for persisting populations in an extension of the Nicholson-Bailey⁶ host-parasitoid

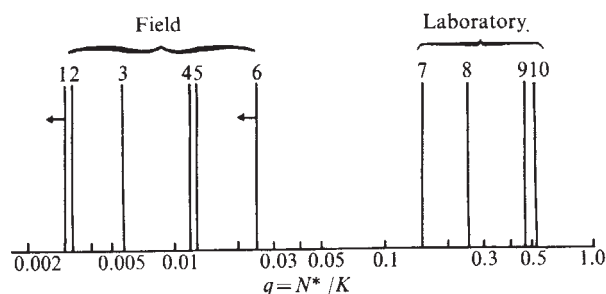


Fig. 1 Field (1–6) and laboratory (7–10) q -values for insect host-parasitoid interactions where q is the ratio of the host population in the presence (N^*) and absence (K) of the parasitoid. The data are (host, then parasitoid): (1) *Aonidiella aurantii*–*Aphytis melinus*⁵⁰; (2) *Chromaphis juglandicola*–*Trioxys pallidus*⁵¹; (3) *Parlatoria oleae*–*Aphytis maculicornis*¹; (4) *Aonidiella aurantii*–*Aphytis melinus*¹; (5) *Operophtera brumata*–*Cyzenis albicans*⁵² (oak-hill site); (6) *Pristiphora erichsonii*–*Olesicampe benefactor*⁵³ (and Ives, personal communication plot 4); (7) *Musca domestica*–*Nasonia vitripennis*⁴¹; (8) *Callosobruchus chinensis*–*Neocatolaccus mamezophagus*⁵⁴; (9) *Anagasta kuehniella*–*Nemeritis canescens*⁵⁵; (10) *Callosobruchus chinensis*–*Heterospilus prosopidus*⁵⁶.

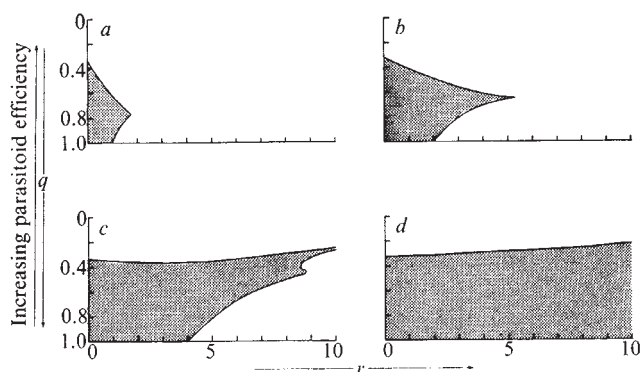


Fig. 2 Varying the duration of the parasitoid generation relative to that of its host (the ratio is denoted by g) affects the stability of the interaction. The shaded areas represent the regions of $r-q$ space which give rise to locally stable equilibria. Note in particular that, although increasing g permits a stable equilibrium for higher values of r , the lowest q which can be attained is almost unaffected. The range of r is extended for illustration beyond that which is biologically feasible; the vertical axis runs from 1 to 0 to represent increasing parasitoid efficiency. The models considered are given in Table 1: a , $g = \frac{1}{2}$, corresponding to model B; b , $g = 1$ (model A); c , $g = 2$ (model C); d , $g \rightarrow \infty$, model D. All four diagrams are drawn to the same scale.

equations of the general form:

$$\begin{aligned} N_{t+1} &= N_t f_1(N_t) f_2(N_t, P_t) \\ P_{t+1} &= P_t f_3(N_t, P_t) \end{aligned} \quad (1)$$

where N_t and P_t are host and parasitoid densities. The simplest version of this equation is model A in Table 1.

The function $f_1(N_t)$ describes the per capita rate of increase of the host as a function of its own density (N_t); $f_2(N_t, P_t)$, the proportional survival of N_t hosts confronted by P_t parasitoids, and $f_3(N_t, P_t)$, the per capita rate of increase of the parasitoid as a function of its own, and its host's, density. Note that in this and most of the subsequent models we have used a simple single-parameter description of the density dependence operating on the host ($f_1(N_t)$); our conclusions are unaffected by using more complex forms that still give rise to a single equilibrium point or cycle⁷. Not surprisingly, however, the effects of introducing forms of $f_1(N_t)$ that generate more than one locally stable equilibrium in the host population⁸ in the absence of the parasitoid are dramatically different. We deal with these after we have explored the simpler density dependence.

Model A is the simplest of host-parasitoid models and its behaviour in relation to the observed field q values is revealing. If a locally stable equilibrium is to be preserved using this model, the biggest impact that a parasitoid can have is to depress the host population to one third the abundance which it maintained in the absence of the parasitoid ($q = 0.33$). For locally unstable (that is, oscillatory but persistent) solutions, the host population fluctuates round an average level which is higher than this. In other words, although model A generates q values comparable to those seen in laboratory populations (Fig. 1), it fails to reproduce the field values by one or even two orders of magnitude.

Obviously, to describe strong host-depression in the field the model must be elaborated. For example, we can question the implicit assumption of model A that the generation times of the host and its parasitoid are equal, because we know that this is incorrect for some of the examples shown in Fig. 1. The effects of varying the relative generation times of the host and its parasitoid on the mathematical models shown in Table 1 (models A–D) and the results of these elaborations are illustrated in Fig. 2. Models B and C are derived from model A by assuming that the host reproduces after an interval of more or less than one parasitoid generation. Thus in model B the host

reproduces after two generations of the parasitoid; in model C, after half a generation. The limit of this sequence, in which the host reproduces continuously (model D), is obtained by integrating a logistic differential equation incorporating parasitism and sampling it at discrete intervals corresponding to the emergence of the parasitoid. The minimum attainable q is hardly affected by these refinements.

Alternatively, instead of using a constant searching efficiency, a , for the parasitoid, we can allow the functional response to vary in several well-documented⁹⁻¹¹ ways. For example, we can allow for the time spent in parasitising a host (handling time t_h) and for the time wasted on interacting with another parasitoid, t_w . A modified attack rate a' incorporating these two phenomena may be written as^{12,13}

$$a' = a/[1 + at_h N_t + bt_w(P_t - 1)] \quad (2)$$

where b is a rate of encounter between parasitoids. The handling time t_h is already known to be unimportant¹⁴. Our analysis of models incorporating this modified attack rate extends this result (see Fig. 3a) and indicates that the depression of the host depends on the ratio of t_w to the total generation time of the parasitoid. This ratio is typically too small by an order of magnitude to be of any importance. Some examples of the resulting minimum q are shown in Fig. 3a, computed using models A and D with the searching efficiency modified according to equation (2).

A third extension is to incorporate a sigmoid functional response^{10,16} in which the parasitoids' efficiency falls off at low host densities. This may be expressed as

$$a' = aN_t/(c + N_t) \quad (3)$$

where c is a parameter which determines the shape of the

response. Contrary to much that has been written about sigmoid functional responses, it is easy to show that in isolation, incorporating equation (3) into the unstable Nicholson-Bailey equations (Table 1, model A, with $K \rightarrow \infty$) cannot stabilise the interaction¹¹; nor does it make any difference to the minimum value for q .

It is not difficult to see why these elaborations are so conspicuously unsuccessful in generating low stable q values. Stability in the simple model (A) is conferred by the density dependent term $f_i(N_t)$ operating on the host; the lower the q value, the weaker this effect will be. The elaborations we have included either do nothing to compensate for the loss of density dependence when the host population is strongly depressed (changing relative generation times; introducing handling times), or are too weak when assigned biologically sensible parameter values (mutual interference); or because of the time delays inherent in the system do too little too late to be effective (sigmoid functional responses).

Such comments apply equally well to models which incorporate combinations of some or all of these elaborations: even the most baroque constructions fail to generate very low stable q values for realistic parameter values.

A check on parameter values

It might be thought that the general class of models specified by equation (1) is so unrealistic that any attempt to match host-depression in the field with the predictions of simple models is doomed to failure. We disagree.

A key parameter in all our models is the dimensionless product $a'K$, which measures parasitoid efficiency. In any model in which $f_i(N_t)$ implies only a single equilibrium in the host population in the absence of the parasitoid, to a good approximation $q \approx 1/a'K$ (where a' is the realised or effective searching efficiency of the parasitoids; see, for example,

Table 1 Summary of the models referred to in the text

Model	Host dynamics are given by the upper equations and parasitoid dynamics by the lower equation of each pair	Notes
A	$N_{t+1} = N_t \exp [r(1 - N_t/K)] \exp(-aP_t)$ $P_{t+1} = N_t [1 - \exp(-aP_t)]$	Basic model; both host and parasitoid have discrete synchronised generations
B	$N_{t+2} = N_t \exp [2r(1 - N_t/K) - aP_t - aN_t(1 - \exp(-aP_t))]$ $P_{t+2} = N_t \exp(-aP_t) [1 - \exp(-aN_t(1 - \exp(-aP_t)))]$	Parasitoid has two discrete generations for each host generation. Both parasitoid generations attack the host. In models B and C the host generation-time is twice as long (B) or half as long (C) as that of the parasitoid. Hence, r and a per generation are scaled accordingly, giving factors of $2r$, $r/2$, and $aP_t/2$ respectively
C	$N_{t+1} = N_t \exp \{r - (rN_t/2K)[1 + \exp(r(1 - N_t/K)/2 - aP_t/2)] - aP_t\}$ $P_{t+1} = N_t [1 - \exp(-aP_t/2)] \{1 + \exp(r(1 - N_t/K)/2 - aP_t/2)\}$	Host has two discrete generations for each parasitoid generation. Both host generations are attacked by the parasitoid
D	$N_{t+1} = N_t \left\{ \frac{r - aP_t}{[r(1 - N_t/K) - aP_t] \exp(-r + aP_t) + rN_t/K} \right\}$ $P_{t+1} = P_t(aK/r)[r - aP_t - \log_e(N_{t+1}/N_t)]$	Host reproduces continuously and is attacked by a parasitoid with discrete generations
E	$N_{t+1} = N_t \exp [r(1 - N_t/K)] [\epsilon K/N_t + (1 - \epsilon K/N_t) \exp(-aP_t)]$ $P_{t+1} = (N_t - \epsilon K)[1 - \exp(-aP_t)]$; for $N_t > \epsilon K$ $P_{t+1} = 0$; for $N_t \leq \epsilon K$	As model A, but the host has a refuge, ϵ , which signifies the proportion of the carrying capacity that is inaccessible to the parasitoid
F	$N_{t+1} = N_t \exp [r(1 - N_t/K)] (1 + aP_t/k)^{-k}$ $P_{t+1} = N_t [1 - (1 + aP_t/k)^{-k}]$	As model A, but the parasitoids search non-randomly, described by a negative binomial distribution with clumping parameter k
G	$N_{t+1} = N_t \exp \{r(1 - N_t/K) - (\beta_{ns} P_{ns} N_t / (N_0^2 + N_t^2)) - aP_t\}$ $P_{t+1} = N_t [1 - \exp(-aP_t)]$	As model A but with complex density dependence operating on the host, permitting more than one equilibrium in the absence of the parasitoid. In this example the complex density dependence is due to polyphagous, non-synchronised background predators with asymptotic maximum attack-rate β_{ns} and abundance P_{ns} . N_0 is a parameter which determines the shape of the response to background predators

N_t and P_t represent the densities of the host and parasitoid populations respectively in generation t . The host has an equilibrium density K in the absence of parasitism and an intrinsic rate of increase r ; it is attacked by the parasitoid at an instantaneous rate a .

equations (2) and (3)). Figure 1 suggests that a necessary (though not sufficient) condition for successful control is that $a'K$ should lie between 40 and 300 ($q \approx 0.025-0.003$). Estimates of $a'K$ are only possible for two of the examples shown in Fig. 1 (*Cyzenis* and *Olesicampe*) but these are consistent with this prediction (Table 2). Table 2 also summarises $a'K$ values for four other host-parasitoid interactions. The results are calculated from field life-tables, with the effective searching efficiency a' estimated per parasitoid generation as the nicholsonian¹⁷ value $\log_e(N_t/N_s)/P_t$. P_t is the density of the searching parasitoids, and N_t and N_s are the densities of hosts entering and surviving the life-history stage which is attacked. The corresponding K values are typical maximum population counts for the appropriate stage in the life-history. Interpretation of these results requires caution, not least because reliable field estimates of the density of searching parasitoids are extremely difficult to make. Nevertheless, we find the agreement between the predicted order of magnitude of $a'K$ and observed values encouraging, and take it to signify that the structure of the model is basically sound.

Spatial heterogeneity

We have concerned ourselves so far with models which assume that the world of the parasitoid and its host is spatially homogeneous. The q values obtained in the laboratory studies shown in Fig. 1 are easily reproduced even by the most simple models (Fig. 3a); the field examples are not. An obvious distinction between the two is the much greater size and physical complexity of field ecosystems compared with their laboratory counterparts, with the corollary that it is the heterogeneity of the field systems which provides an explanation for their low q values. We now proceed to examine this hypothesis.

Models can be used to mimic a spatially heterogeneous environment in two ways. One approach is to describe explicitly what happens in n different homogeneous patches ($n > 1$) and then consider how animals move between patches^{10,18,23}. Such models tend to be complex, with an inevitable proliferation of parameters. The second approach implicitly incorporates spatial heterogeneity through the modifying effects it must have on the rate at which the parasitoid encounters its host. We have used this approach in two ways: in the first case by incorporating a host refuge, and in the second case by considering how the exploitation of patchily distributed hosts modifies the parasitoid's attack-rate, a process which we have elsewhere termed 'pseudo-interference'¹⁵.

The way in which a prey refuge has been incorporated into the model is shown in Table 1 (model E). Useful reviews of related models with different biological details are provided by Murdoch and Oaten¹⁰ and Hassell and May¹⁴. Here we have assumed that some small fraction of the carrying capacity of the

hosts is unavailable to the parasitoid in each generation, but that all hosts, whether in the refuge or not, compete for some limiting resource set by K . With this simple modification low values for q become feasible. A typical example is shown in Fig. 3b. Clearly, this result applies only to a small refuge. If 10% of the hosts are unavailable, then the minimum q can never be less than 0.1. The possible existence and nature of small host refuges in cases of successful biological control deserves further examination²⁴. Note that a refuge need not be regarded as a fixed, finite entity. In a large, complex universe it is likely that patches of hosts will remain undetected for a time, and this random element in the discovery of new host-patches by the parasitoids effectively constitutes a refuge.

The second approach to spatial heterogeneity considers parasitoids distributing themselves in an aggregative manner according to a patchily distributed host, when differential exploitation of host patches occurs. Models which explicitly include a refuge may be viewed as an extreme case of this phenomenon. It may be shown that such differential exploitation produces a decline in parasitoid efficiency with increasing parasitoid density. This effect, which we have termed pseudo-interference¹⁵, cannot readily be distinguished from genuine mutual interference in which the decline in efficiency is due to encounters between searching parasitoids. An empirical model of this process proposed by Hassell and Varley²⁵ has efficiency a' declining with parasitoid density. This can be described by:

$$a' = a(P)^{-m} \quad (4)$$

where m measures the extent of mutual interference. (Note that the Hassell and Varley model is dimensionally incorrect; equation (4) corrects this without in any way altering their conclusions.) By considering underlying models of parasitoid behaviour we have shown elsewhere¹⁵ that real interference between individuals will lead at most to values of m of about 0.1. (See also ref. 11.) However, where parasitoids respond to patchily distributed prey, empirical fits to data which ignore this spatial heterogeneity may result in m values as high as 0.8, almost all of which is attributable to pseudo-interference. The effect of such m values on the minimum q attainable is illustrated in Fig. 4. Clearly the stabilising effect of the parasitoids' response to host distribution is marked, and low q values are now easily generated.

An alternative way of introducing spatial heterogeneity is due to May²⁶. In a spatially homogeneous environment the function $f_2(N_t, P_t)$ can be considered as the zero term of a Poisson process involving some modified rate of encounter between randomly searching parasitoids and their hosts^{6,27}. May's alternative is to consider $f_2(N_t, P_t)$ as the zero term of an

Table 2 Estimates of parasite efficiency, $a'K$, for six field population

Parasitoid	Host	$a'[m^{-2} \text{ generation}^{-1}]$ in field*	Approximate host upper population levels $K(m^{-2})^{*†}$	$a'K$	Ref.
<i>Apanteles fumiferanae</i>	<i>Choristoneura fumiferana</i> Instar I & II	0.057 ± 0.021 ($n = 5$)	1,000	57	57
<i>Cyzenis albicans</i>	<i>Operophtera brumata</i> larvae	0.116 ± 0.04 ($n = 12$)	1,000	116	17, 52
<i>Cratichneumon culex</i>	<i>Operophtera brumata</i> pupae	0.157 ± 0.04 ($n = 17$)	870	137	17
<i>Glypta fumiferanae</i>	<i>Choristoneura fumiferana</i>	0.143 ± 0.043 ($n = 12$)	1,000	143	59
<i>Olesicampe benefactor</i>	<i>Pristiphora erichsonii</i> Instar II	2.65 ± 0.97 ($n = 6$)	100	265	53
<i>Carcelia obesa</i>	<i>Bupalus piniarius</i> Instar II & III	1.52 ± 0.27 ($n = 6$)	200	303	17, 60

*Values are means $\pm$ s.e.m.

†High levels of host abundance cause serious deflation and may not be sustainable; the data therefor represent an upper bound to K .

aggregated distribution, for example the negative binomial. This modification (model F, Table 1) again generates low values for q for sensible parameter values (Fig. 3b) and provides an important complementary model of the effects of spatial heterogeneity.

These analyses have none of the complexity of several recent studies which model spatial heterogeneity in predator-prey systems^{10,18-23}. We believe, however, that the three simple models capture the essence of the problem, which is the differential exploitation of patches of hosts in different parts of the environment. Between them they provide a convincing demonstration of the potential importance of spatial heterogeneity in generating low, stable q values. Interestingly, however, they are not the only mechanism which may be involved.

Complex forms of density dependence operating on the host

We now return to models which treat the world as being spatially homogeneous, and choose instead to vary the form of the host density dependence function $f_i(N_i)$.

Since Takahashi²⁸ there have been several studies^{29,30} which have focused on the way in which background predators or parasitoids (absent in the laboratory populations shown in Fig. 1) may produce an effective functional form of density dependence that can possess two locally stable equilibria: a lower one produced and stabilised by natural enemy-induced mortality and an upper one, by resource limitation. May provides a detailed review⁸. A lower equilibrium can be maintained by polyphagous predators (for example, birds, ants or spiders) which are only very loosely coupled to the host population in question. The resulting lower equilibrium is not globally stable, and following a perturbation the host may 'escape' to the upper one.

Introduction of a synchronous parasitoid to this system results in the destabilisation of the upper equilibrium and a population trajectory that takes the host down to its lower equilibrium. (Fig. 3b; model G, Table 1.) Providing the parasitoids show some degree of mutual interference (however slight) or alternatively there is some aggregation in response to a variable host density, they persist in the system; otherwise model G predicts that they become extinct.

This model provides an alternative hypothesis to explain successful biological control. The mechanism postulated is totally different from that envisaged in the previous section and has very different implications for management. In the final section we attempt to assess the relative importance of these two alternatives, and their implication for the choice of suitable natural enemies.

Conclusions and discussion

Of the 120 attempts at biological control listed by DeBach³¹ approximately one third (42) were completely successful. Most of these are probably similar in their degree of host-depression to the conspicuous successes shown in Fig. 1. A further 48 were classified as 'substantial successes' and 30 as 'partial'; q in this latter group is presumably high. Obviously, a field environment does not necessarily imply that q is low for a host-parasitoid interaction, although the reverse seems to be true.

Our analysis shows that high (> 0.3) field and laboratory values for q are easily reproduced by the simplest models. (This does not imply that in these systems the parasitoids necessarily behave in the simple way that model A suggests; merely that more complex forms of parasitoid behaviour are not necessary to explain the persistence of the interaction.) In marked contrast, very low (field) q values can be generated only by a limited range of models, involving two very different mechanisms, which for convenience we refer to as 'spatial heterogeneity' and 'complex density dependence'. Evidence of two sorts suggests that the former is much more important than the latter.

First, models incorporating spatial heterogeneity without complex density dependence predict that q should be of the

order of $1/a'K$. On the other hand, if complex density dependence were to be the key, the lower equilibrium population size of the host would be determined largely by background mortality, and hence would be independent of the efficiency a' of the introduced parasitoid. In other words, there would then be no reason for q to be of the order of $1/a'K$. The available evidence, although limited, indicates that realised q values in successful biological control programmes are indeed close to $1/a'K$ and hence that strong host depression is not usually produced by complex density dependence.

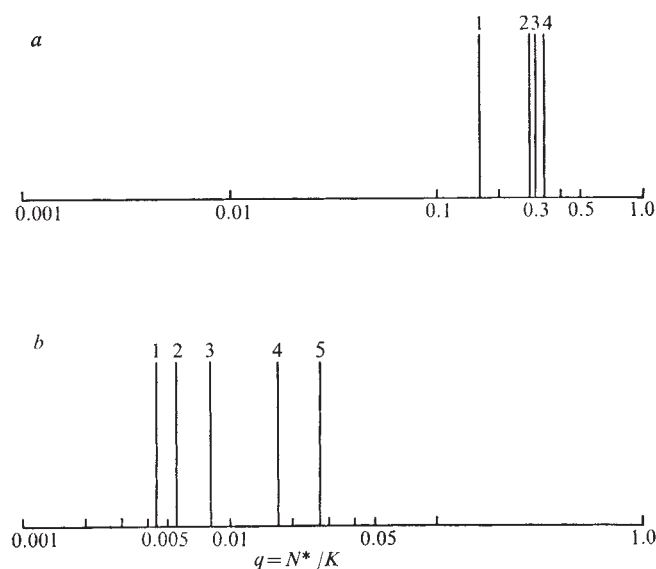


Fig. 3a. The vertical lines indicate the smallest fractions, q , of the carrying capacity to which a host population can be depressed in a stable interaction with a synchronous parasitoid, according to the spatially homogeneous models A–D listed in Table 1. None of these models, even with elaborations, is capable of reproducing the low values of q illustrated in Fig. 1, which are observed in cases of successful biological control. The basic models A, B and C all have a minimum q at 0.33 (line 4), obtained as $r \rightarrow 0$; model D with continuously reproducing hosts permits a slightly lower value (line 2) at $r = 5$. The elaborations given in equations (2) and (3) have been studied for models A and D. It can be shown that the minimum q can be reduced only for non-zero $P^*(\partial a' (N_i, P_i) / \partial P_i)^*$, where the partial derivative is evaluated at equilibrium. Therefore the inclusion of a sigmoid functional response or of handling time (or, in general, of any functional response for which a' is a function of N_i only) does not affect the result. Mutual interference, however, does enable q to be reduced, but the decrease is small even for extreme¹⁵ values of the parameters. Models A and D yield lines 3 and 1 respectively for $bt_w/(aT) = 0.1$, again independently of handling time. **3b.** In contrast, those models which attempt to describe the aggregation of parasitoids on a spatially heterogeneous host population can readily generate values for q comparable with the lowest shown in Fig. 1, for values of $a'K$ similar to those listed in Table 2. The low q values are approached, but not reached, by the only spatially homogeneous model in this sequence, model G, in which the host is assumed to exhibit a complex form of density dependence due to background predation. For comparability q is defined as the ratio of the lower to upper single-species equilibria. The effective q is illustrated by line 5 for $r = 0.5$ (as in all subsequent examples), $\alpha = N_0/K = 0.06$, $\gamma = \beta_{ns} P_{ns}/rK = 0.25$. The upper equilibrium is unstable for $aK \gtrsim 1.1$. The remaining models illustrated have a simpler form of density dependence but include some representation of heterogeneous environment. A simple model with an explicit refuge of 1% of the carrying capacity, model E with $\epsilon = 0.01$, yields the q shown by line 4 for $aK = 434$, $a'K = 74$. A general model of spatial heterogeneity incorporating moderate pseudo-interference ($m = 0.21$ in model A modified according to equation (4)) allows stable equilibria for q down to below 0.01 (line 3), compared with $q = 0.33$ for $m = 0$. A stronger aggregative response permits a stable equilibrium for arbitrarily small q , the particular value depending on $a'K$. The example shown (line 2) is for $m = 0.4$, $aK = 145$, $a'K = 230$. May's model of aggregated parasitoid attacks (model F) similarly allows arbitrarily small q . The example (line 1) is for $k = 0.5$, $aK = 520$, $a'K = 300$.

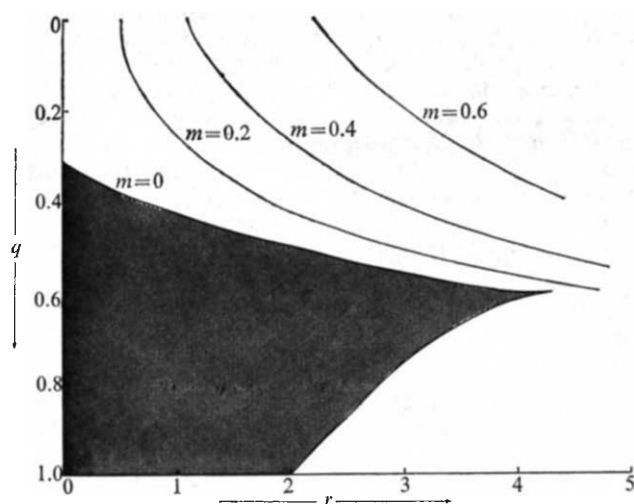


Fig. 4 Increasing the aggregative response of a parasitoid on a patchily distributed host population, interpreted here as increasing pseudo-interference, makes low values of q more readily attainable. The shaded region shows the stability properties of the basic model (model A in Table 1): this is identical to Fig. 2b. For $m > 0$ (model A modified according to equation (4)) the upper stability boundary moves to enlarge the stable region, so that, for example, $q \rightarrow 0$ is stable for $m = 0.4$ up to $r = 1.1$. The lower boundary changes only very slightly with m .

The second body of evidence concerns the types of parasitoids that make good control agents. The spatial heterogeneity hypothesis suggests that such parasitoids have high aggregative responses^{9,11,14} to host patches, high powers of dispersal between patches, and high effective searching efficiencies (since $q \approx 1/a'K$). These are undoubtedly characteristics of the parasitoid in several successful control programmes^{1,11}. The impact of *Cyzenis* on the winter moth *Operophtera* in Canada is a good example^{11,17,32}. On the other hand, if complex density dependence ultimately provides the control, high $a'K$ and high aggregative ability are no longer critical. This does not accord with experience. Thus parasitoid efficiency (a') declines with increasing polyphagy³²⁻³⁴ and lack of synchrony with the host³⁵, and in general neither poorly synchronised nor polyphagous parasitoids make good control agents^{1,11,32,34}.

The available evidence therefore suggests that spatial heterogeneity, the patchy distribution of the host and the differential exploitation of these patches by the parasitoid provide the key to most cases of successful biological control. This conclusion conforms to the biological intuition of a number of workers over a long period³⁶⁻⁴¹, the most perceptive and detailed summary being that of Huffaker, Messenger and De Bach⁴². However, both mechanisms remain as possibilities.

This study generates several important questions which must be answered if biological control of insect pests by introduced parasitoids is to become less empirical. First, there is the obvious need to determine whether complex density dependence has been a factor in any successful control programmes. Second, studies are needed on the efficiencies of parasitoids in cases of both successful and unsuccessful control. Third, the spatial distributions of selected pest species, the responses of their parasitoids to these distributions and hence levels of pseudo-interference should be investigated in the field. The first of these questions is particularly important because the management implications of the two alternative ways of generating low q values are profoundly different. Under the complex density dependence hypothesis any lowering of the efficiency of the polyphagous predators by altering the management regime may result in an outbreak of the pest as the system moves from the lower equilibrium to the higher. Indeed one of the characteristics of control by this mechanism is likely to be the occurrence of

periodic outbreaks of the pest due to natural fluctuations in the amount of background mortality. In contrast, if control is due to the parasitoid's response to host patches, only strongly selective killing of the parasitoid will result in an outbreak. Hence the resilience² of the system with polyphagous predators is considerably less than that with only parasitoid control.

Our models highlight several characteristics of parasitoids that are likely to render them efficient control agents. Thus high effective attack rates and a high egg complement are clearly important. These will be facilitated by the synchronous emergence of the parasitoid with appropriate life-history stages of the host, lack of polyphagy, and less important, a low handling time. All these points have been made previously. However, it has not been appreciated that the relative generation times of the host and its parasitoid are unimportant, nor that the high efficiency of the chosen parasitoid must be complemented by an aggregative response to the pest's distribution which will produce sufficient pseudo-interference to stabilise the interaction. These characteristics of the parasitoid are also desirable even if complex density dependence could also stabilise the interaction at a low q value.

Finally, we can deal briefly with predators. Predator-prey models in which the predators are monophagous and have discrete generations give rise to the same paradox as host-parasitoid models; namely that where control is successful⁴³⁻⁴⁷, prey are depressed more than can be predicted by spatially homogeneous models². Indeed the minimum predicted values for q are even higher (approximately 0.5) than in host-parasitoid interactions. Unfortunately, we cannot carry out such a detailed analysis for predators and their prey because their interactions need more parameters to describe them, and we can only guess what values some of these take in the field. However, it does not seem unreasonable to suggest that the principles outlined in this paper will apply wherever exploited populations are strongly depressed by a natural enemy, be they host-parasitoid, predator-prey or plant-herbivore^{1,49} interactions in artificial or natural ecosystems.

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letters to nature

Gravitation, primordial stars and the dark mass

THE possibility that the gravitational constant G decreases as the Universe expands is a subject of continuing theoretical and experimental interest. Such a cosmology is used here to explain the low luminosity of the 'missing mass' in clusters of galaxies, and the 'heavy haloes' of spiral galaxies, in terms of the rapid initial evolution of a generation of primordial stars.

The velocity dispersions of clusters of galaxies imply virial masses much larger than the combined mass in the visible galaxies^{1,2}. Most of the mass of a rich cluster evidently consists of matter between the galaxies with mass-to-light ratio (in solar units) $M/L \gtrsim 300$, exceeding M/L for the visible matter (mainly elliptical and SO galaxies) by a factor >10 . For spiral galaxies, rotation curves³ and stability arguments⁴ imply the presence of heavy haloes with more mass than the disk but very little luminosity. I shall refer to heavy haloes and the missing mass collectively as the 'dark mass'.

Rees⁵ has suggested that much of the cosmic material might go into a generation of primordial stars (Population III) formed shortly after recombination, at $(1+z_p) \simeq 300$. These stars could provide an explanation of the dark mass if they all have masses $M \lesssim 0.3 M_\odot$; but Rees argues that the initial mass function of Pop III is likely to be similar to that for more recent populations. I suggest that Pop III is depleted in living, massive stars because of accelerated stellar evolution due to a large value of G when the Universe was young.

Homology relationships⁶ give $L \propto G^7$ for $M \approx M_\odot$, so that a star exhausts its nuclear fuel and ages more quickly for larger G . This leads to an evolutionary age for a star born at t_1 ,

$$\Delta t_{ev} = \int_{t_1}^{t_0} (G/G_0)^7 dt = \frac{t_0}{(7n-1)} \left[\left(\frac{t_0}{t_1} \right)^{7n-1} - 1 \right]$$

where t_0 is the present age of the Universe, and we assume $G = G_0(t/t_0)^{-n}$. If the scale factor obeys the usual equation $R^2\ddot{R} + (4\pi/3)G\rho_0 = 0$, then a power law solution exists, $R = (t/t_1)^{(2-n)/3}$, for ρ_0 equal to a critical density $\rho_c(H_0, n)$ analogous to that for the Einstein-deSitter Universe. I assume this solution to show the effect of variable G on the primordial stars.

Table 1 gives t_1 and the main sequence turnoff mass M_T for stars born at $1+z = R^{-1} = 300$, for several values of n . (In Table 1, $h \equiv H_0/100 \text{ kms}^{-1} \text{ Mpc}^{-1}$.) M_T was calculated assuming main sequence lifetimes $\tau_{ms} = (13,000 \text{ Myr})(M/M_\odot)^{-2.5}$ for $G = G_0$. Even a gentle variation of G , $n = 0.2$, gives a main sequence turnoff of only $\sim 0.2 M_\odot$, rather than $M_T \approx M_\odot$ for $G = \text{constant}$. M/L for Pop III is estimated as follows. For a Salpeter⁷ initial mass function $dN/dM \propto M^{-2/3}$, M/L for the whole population varies roughly as M/L for the individual stars at the main sequence turnoff, which in turn is proportional to Δt_{ev} . (The dependence on the lower mass limit is weak.) We may therefore ratio L/M for the primordial stars to that for a typical E galaxy as

$$\frac{(M/L)_p}{(M/L)_E} \approx \frac{(\Delta t_{ev})_p}{(\Delta t_{ev})_E} \quad (2)$$

Dynamical arguments⁸ suggest that ellipticals formed late enough to experience little excess evolution for the small values of n considered here, so we may take $(\Delta t_{ev})_E \approx t_0$. Table 1 gives the resulting values of M/L for Pop III for an assumed⁹ $(M/L)_E = 30$. For $n \gtrsim 0.2$, Pop III has $M/L \gtrsim 10^3$, sufficiently high to explain the dark mass.

Although a value $H_0 = 50$ is now widely accepted¹⁰, there is evidence^{11–14} that a value $H_0 = 100$ should be seriously considered. For $H_0 = 100$, one has $t_0 \leq 9,800 \text{ Myr}$; and the Universe is younger than the evolutionary ages of globular clusters¹⁵, for example, $\Delta t_{ev} = 15,000 \text{ Myr}$ for M15. This discrepancy can be removed if G varies, as pointed out by Dicke⁶. This is illustrated by Table 2, which gives the true and evolutionary ages of globular clusters and Pop III for a cosmology, with $H = 100$, $n = 0.2$, and $\rho_0 = \rho_c$. A further result is that the true age difference between 47Tuc and M15 is brought into

Table 1 Mass-to-light ratio of primordial stars

n	ht_1 (10^9 yr)	ht_0 (10^9 yr)	$h\Delta t_{ev}$ (10^9 yr)	$h^{-0.4}M_T$ ($M_\odot$)	$h(M/L)_T$	$(M/L)_p$
0.0	1.25	6.53	9.8	1.12	0.75	30
0.1	0.76	6.21	30.0	0.71	2.4	93
0.16	0.55	6.01	103.0	0.44	7.8	510
0.2	0.44	5.88	650	0.21	49.0	3×10^3
0.25	0.33	5.72	9×10^3	0.074	670.0	5×10^4

Table 2 True and evolutionary ages of ancient stars

Object	Δt_{ev}	Δt^*	t_1	$1+z_1$
47 Tuc ¹⁵	11.5	4.5	1.4	2.0
M15 ¹⁵	15.0	4.9	1.02	2.9
Pop III	~650	5.9	$\sim 4.4 \times 10^{-4}$	300

*True age = $t_0 - t_1$ (All ages in 10^9 yr)

agreement with the free-fall collapse time of the galactic halo. (There is, however, no compelling evidence for a rapid collapse. Moreover, an alternative way to reconcile the cluster age spread with the halo collapse time is to assume that the mass in the disk and in the heavy halo was still outside the Pop II halo when the latter collapsed. If the Pop II halo contained a fraction $10^{-1\epsilon}$ of the current mass of the Galaxy, then the halo collapse time was a factor $\approx 3\epsilon^{-1/2}$ longer than the current halo crossing time. A case for such a sequence of events can be based on the mean tidal angular momentum induction calculated by Gott⁸ and the scenario for heavy halo formation given by Gunn¹⁶.)

Pop III has $M/L \approx 500$ for $n = 0.16$; and this implies $(-G/G) \approx (1.2 \times 10^{-11}) (h/0.5) \text{ yr}^{-1}$. This is consistent with experimental limits from radar ranging¹⁷, lunar occultations¹⁸, and geophysics¹⁹. The Brans-Dicke²⁰ scalar-tensory theory gives $n = 2/(3\omega + 4)$; Solar System experiments^{21,22} imply $\omega > 29$ and hence $n < 0.02$. Therefore, a different theory of gravity is required to explain the dark mass.

Strong evidence for a variable G would be provided by observation of a population of stars with evolutionary ages unambiguously greater than H_0^{-1} , which might be called 'hyper-evolved stars'. For $H_0 = 50$, this requires $\Delta t_{ev} > 20,000$ Myr. Observation of Pop III stars in the Galaxy with $M_T < 0.7 M_\odot$ would suffice. The detection²³ of an extensive red halo around NGC4565 may be a step in this direction. However, the observed light could be dominated by a small fraction of the Pop III stars formed later than most when G was smaller and stellar evolution less rapid; in this case the bulk of Pop III has Δt_{ev} even larger than for the observed stars. One possibility is that some dwarf spheroidal galaxies formed sufficiently early to be hyperevolved. W Virginis stars show a progressive decrease in period from M31 to the globular clusters to the dwarf spheroidals²⁴. Although the interpretation is complicated by composition differences, one possibility is that the progression is one of age. If so, the dwarf spheroidals may have evolutionary ages $\sim 5,000$ Myr greater than the globulars (Iben, personal communication); and the dwarf spheroidals are hyperevolved even for $H_0 = 50$.

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Coronal holes at 11.5 and 21 cm observed with the Arecibo radio telescope

CORONAL holes have been seen in several regions of the electromagnetic spectrum. Observations have been made in the X-ray and UV (ultraviolet) regions by several groups^{1,2}. These observations, however, can be made only from space and are not always available. Coronal holes have also been identified from spectroheliograms made from the ground³ at the 10,830 Å line of He. The 10,830 Å spectroheliograms are now obtained on a regular basis at Kitt Peak. In the radio domain several groups have attempted to identify coronal holes. These include observations at 80 and 160 MHz with the Culgoora radio heliograph⁴, at 10.7 GHz with the Bonn 100 m antenna⁵, and at 85 GHz with the NRAO 11 m antenna⁶. Our observations reported here made at 1,420 and 2,600 MHz with the 1,000 ft Arecibo radio telescope show the presence of coronal holes very clearly and provide for the first time an excellent matching of coronal hole images obtained from decimetre solar radio maps and 10,830 Å spectroheliograms.

Our solar radio maps at 21 cm (1,420 MHz) and at 11.5 cm (2,600 MHz) using the Arecibo radio telescope were obtained between 31 August and 4 September 1977. The half power beamwidths, with the feeds used in these observations, were 3.8 arc min at 21 cm and 3.3 arc min at 11.5 cm. Solar radio maps were obtained at both wavelengths by making 21 scans in right ascension (RA). The scans were separated by 2.0 arc min in declination (dec) with each scan being 60 arc min in RA. From these scans a grid of antenna temperatures was constructed with the points of the grid spaced 2.0 arc min apart in both RA and dec. The solar radio maps shown here were obtained by contouring such a grid, which was centred on the centre of the solar disk.

Between 31 August and 4 September, the Arecibo radio telescope can observe the Sun for less than two hours near transit. Hence when a drift scan process was used, only one map of 21 scans could be made per day because each scan took 4 min. We, therefore, had to choose one of the two available wavelengths for that day. We found, however, that we could also obtain maps at both wavelengths on a single day by driving the antenna at twice the sidereal rate, thus spending only 2 min for each scan line, while executing a boustrophedonic raster.

During our five day observation period we obtained seven maps: two on 31 August 1977 (11.5 and 21 cm); one on 1 September 1977 (21 cm); one on 2 September 1977 (11.5 cm); one on 3 September 1977 (11.5 cm); and two on 4 September 1977 (11.5 and 21 cm). During the observations we were concerned that by driving the antenna we were introducing some additional noise because of small pointing fluctuations of the moving antenna. Subsequently, however, we found that the solar radio maps obtained from driven scans were as good as those obtained from drift scans.

The sensitivity of the antenna temperatures was approximately 0.2% of the antenna temperature corresponding to a quiet region on the solar disk. In making the solar maps, the antenna temperature range was scaled by setting the antenna temperature of the quiet solar disk equal to 100 and the antenna temperature away from the Sun equal to 0. From each grid of antenna temperatures we made several contour maps in an effort to find the contour scheme that

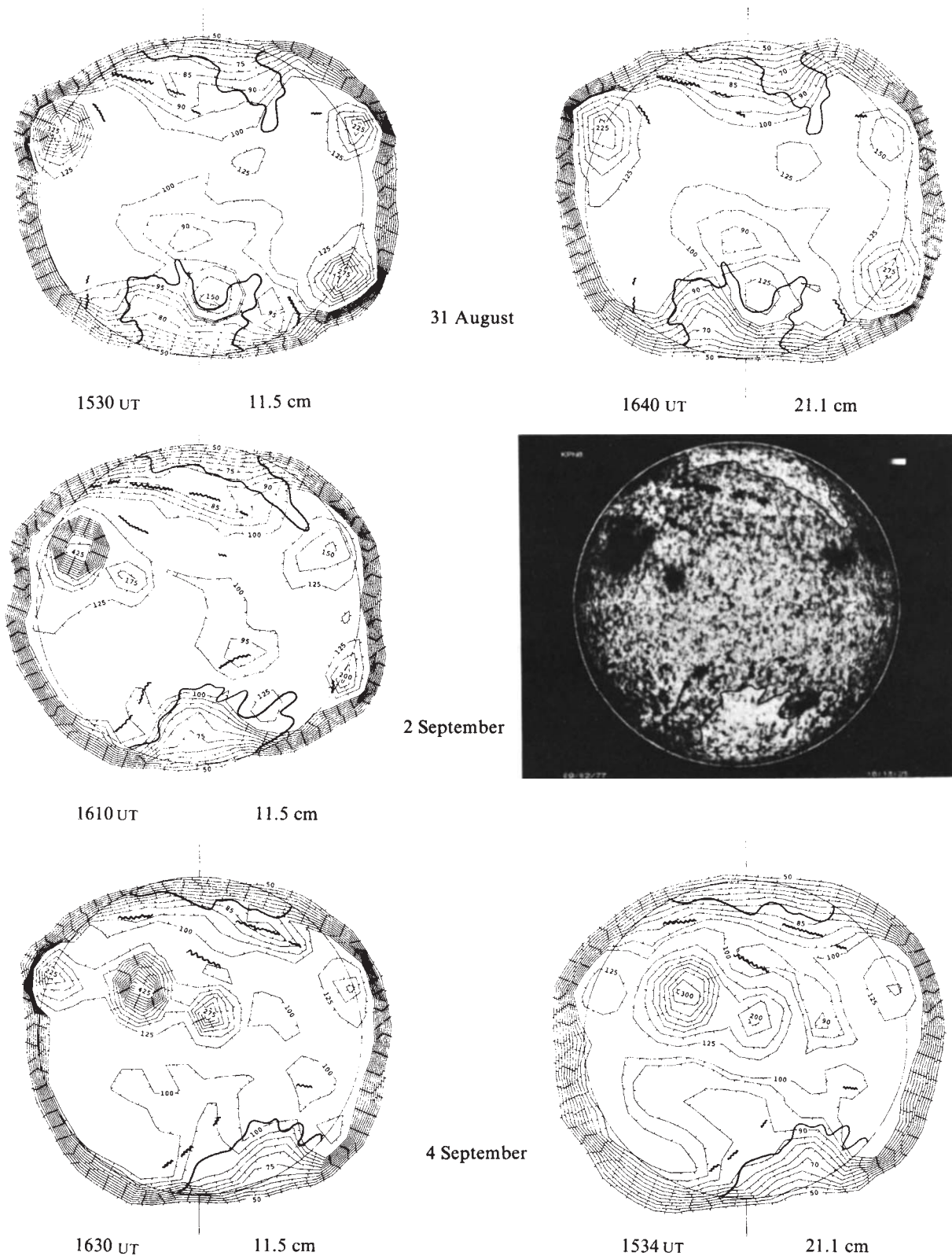


Fig. 1 Solar radio maps at 11.5 and 21 cm obtained with the Arecibo radio telescope showing the position and rotation of a large coronal hole in the southern hemisphere between 31 August and 4 September, 1977. The boundaries of the coronal holes were obtained from 10,830 Å spectroheliograms from Kitt Peak, one of which is shown above for 2 September, 1977.

would best represent the coronal hole region. The solar maps shown in Fig. 1 were constructed as follows: in the temperature range 50–100 the contour interval was set equal to 5 to show clearly the coronal hole region which is

included in this temperature range. At temperatures above 100, however, the contour interval was set equal to 25 to accommodate the active regions which can reach antenna temperatures several times the quiet solar disk background.

Special tick-marks have been placed on the low temperature side of the contours to facilitate the reading of the maps.

Figure 1 shows the solar radio maps obtained on 31 August 1977, 2 September 1977, and 4 September 1977. On 31 August and 4 September maps had been made both at 11.5 and 21 cm, while on 2 September we had a map only at 11.5 cm. Thus, for 2 September we have included the 10,830 Å spectroheliogram from Kitt Peak which shows the two coronal hole regions, one in the north polar region and one in the south, which were present on the solar disk during the period of our observations.

The perimeter of each coronal hole was copied directly on to our radio maps by making the photospheric disk of our maps the same size as the solar disk of the spectroheliograms. The coronal holes appear on the Kitt Peak spectroheliograms as bright regions; dark areas, however, correspond either to active regions which on the radio maps are hotter than the solar disk background, or to filaments which on the radio maps appear as regions cooler than the solar disk background (Straka *et al.*⁷).

Because both filaments and coronal holes show up on the radio maps as regions of lower brightness than the quiet solar disk, we also obtained H α -pictures for the same period from the Sacramento Peak Observatory. The H α -pictures, which show the filaments present on the solar disk, were used to draw these filaments on our radio maps. From the radio maps of Fig. 1 and the superimposed coronal hole boundaries from the Kitt Peak spectroheliograms, the coronal hole in the southern hemisphere is very clearly seen at both wavelengths. These are among the best radio images of coronal holes obtained at any wavelength by any radio telescope.

The coronal hole in the north polar region, on the other hand, is not seen very clearly on the radio maps because it is considerably smaller, it is confined near the solar limb, and there is a large filament next to it. Under these adverse conditions it is not easy to identify a coronal hole from the radio maps alone, while under normal conditions, as with the coronal hole in the southern hemisphere, a coronal hole may be readily identified on the solar radio maps.

Measurements obtained from these radio maps show that the antenna temperature in the coronal hole region is 20–25% below the solar disk background at 11.5 cm, and 30–35% at 21 cm. These results support the conclusions of a preliminary analysis of the radio spectrum of Coronal Hole 1 (ref. 8) which indicate that the depression, relative to the solar disk background, produced by the coronal hole becomes progressively more pronounced as we move from 4 cm to longer wavelengths in the decimetre range.

The observations reported here are valuable because: (1) They have demonstrated that in the radio domain coronal holes are most pronounced, relative to the solar disk background, in the decimetre wavelength range; provided, of course, that an instrument of sufficient sensitivity (~1% of solar disk background) and angular resolution (3–4 arc min) is available.

(2) They have demonstrated the unique value of the 1,000 ft Arecibo radio telescope for coronal hole studies, especially because Arecibo had never been used before for making solar radio maps. In a sense we have uncovered a very special antenna-wavelength combination for the study of coronal holes.

(3) They have demonstrated that the coronal hole images obtained from the Kitt Peak 10,830 Å spectroheliograms and the Arecibo 11.5 and 21 cm solar radio maps are in excellent agreement. This result strengthens the reliability of both processes, which is significant because these are the only two methods available for continuous coronal hole observations from the ground.

Solar radio maps in the decimetre range with the Arecibo radio telescope therefore hold great promise for the study of

the spatial and temporal development of coronal holes, their correlation with solar wind and geomagnetic data (Krieger *et al.*⁹, Sheeley, *et al.*¹⁰) and in the construction of plasma density and temperature profiles inside the coronal hole region (Dulk *et al.*¹¹).

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Propagating inhomogeneities in the dust tail of comet West 1975

COMET West has displayed some remarkable properties. Sekanina¹ has studied the multiple splitting of its nucleus and also discussed the strange structure of its dust tail. This tail is characterised by a system of bright bands which do not correspond to the true synchronous band² and which are shown by very few comets. We call these striae (striated tail) and to study them we have examined numerous original and duplicate pictures, and selected four observations (Table 1) which reveal that the morphology was relatively well conserved during the evolution of the dust tail over a period of more than 4 days. We have identified three striae (Fig. 1) which seem to propagate in space while retaining their basic form. This phenomenon is

Table 1 Parameters of the observations considered

Dates (March 1976)	Focal length of objective (mm)	Earth- comet distance	Sun- comet distance	Observers
3.2	500	0.811	0.324	L. Pansecchi
4.18	55	0.822	0.350	P. Stättmay
5.50	50	0.837	0.386	D. Elmore
7.50	55	0.864	0.441	S. Koutchmy

not unusual in plasmas, such as gas tails of comets³ or streamers in the solar K-corona^{4,5}. However, this is apparently the first time that it has been observed in dust 'clouds'. These striae, which we describe here, are made of dust grains which can be readily demonstrated by the wide-field colour photographs made by comet observers: the gas tail (type I) appears distinctly blue because of CN emission and is directly in the anti-solar direction as well as being well separated from the large dust tail (type II) which has a neutral colour.

To study the dynamic behaviour of these striae, prints were obtained on the scale of and superimposed on the Becvar Sky Atlas, taking into account the orbital motion of the comet

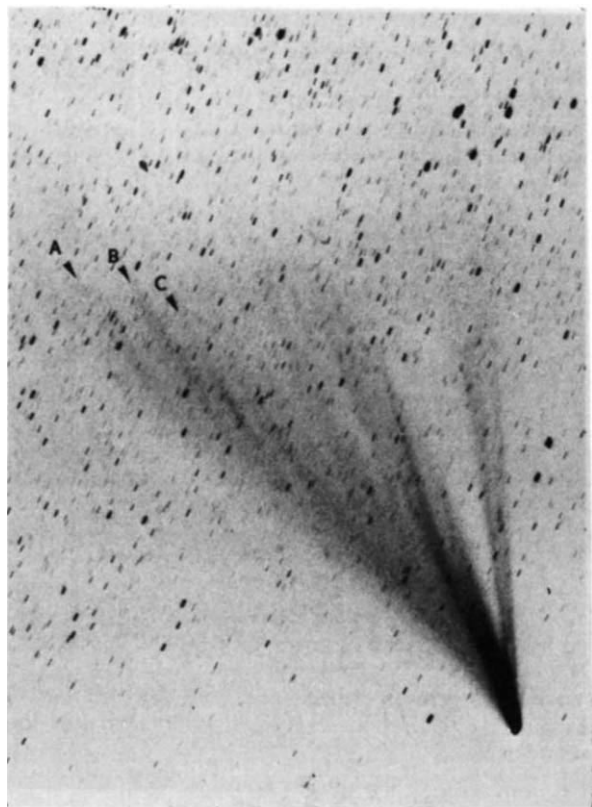


Fig. 1 Print of a colour picture of comet West 1975 n on 7.56 March obtained with a blue filter ($\lambda_{\text{eff}} = 4,700 \text{ \AA}$). The characteristic features of the dust tail here studied are marked A, B, and C; the gas tail (at right) appears enhanced due to the filter as expected.

(Fig. 2). We tried to reconstruct the trajectory of the striae assuming a linear displacement as projected on to the sky and making a point-to-point correspondence between the four position of each stria. It is then possible to derive an estimate for the apparent displacement, and for the corresponding velocities, V , neglecting: the variation of V between two nearby locations ($\Delta V/V \ll 1$); the variation of the comet–Earth distance (about 10%); the variation of the angle between V and the sky plane over the time span considered here (typically 22°); any intrinsic variations due to the change of morphology of each stria—this is the main source of error in our method.

Figure 3 shows the resulting values. Assuming that the bulk of dust making these striae was ejected at perihelion, as suggested by the infrared observations of Ney and Merrill⁶ of a burst between 24.8 and 25.8 February, we tried to find the law of variation of V . The data points are poorly compatible with a linear function, $V = \alpha t + \beta$, while a quadratic one $V = \alpha t^2 + \beta t + \gamma$ offers a more satisfactory agreement.

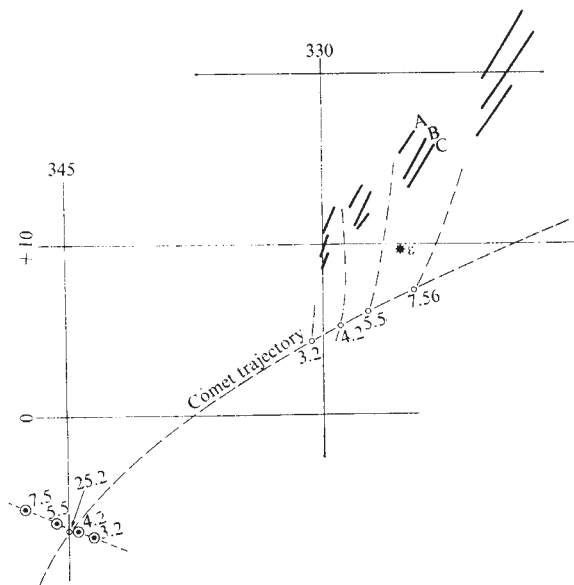
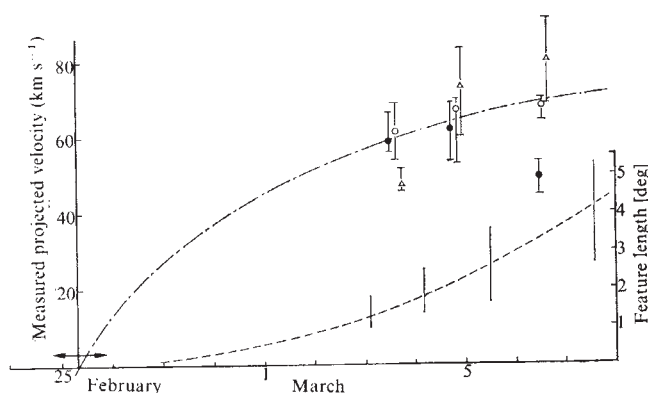


Fig. 2 A portion of the celestial sphere (right ascension and declination are given with respect to the 1950.0 positions) with the location and the shape of the observed features. The numbers correspond to the times of observation and the corresponding positions of the nucleus ($\odot$) and of the Sun ($\odot$) are indicated as well as their trajectory in the sky plane. The dashed-dotted lines show the eastern edge of the dust tail. Note the location of the bright fiducial star ϵ , Pegasi.

After accounting for the projection effect, we found an initial total acceleration of 0.34 m s^{-2} which can be compared with the value of gravitational acceleration at perihelion of 0.154 m s^{-2} . A preliminary analysis using the synchrone-syndyne method⁷ has confirmed that the striae lie in regions corresponding to extremely large values of the ratio β of the radiation pressure force to the gravitational attraction, ranging approximately from 0.6 to 2. These values are not compatible with silicate ($\beta \lesssim 0.6$ (ref. 8)); and would imply the exclusive presence of conducting materials (iron, magnetite, or graphite?) with a narrow size distribution peaked at $0.1 \text{ \mu m}$. This situation seems very unlikely and contradicts the conclusions of Ney and Merrill⁶ who identified the silicate signature. It is, therefore, necessary to look for new interactions acting upon these grains which should additionally explain why striae exist and why they retain

Fig. 3 Time variations (scale in days) of the measured projected velocities of displacements of the A (Δ), B ($\circ$) and C ($\bullet$) features (striae); the bars indicate the full dispersion of measurements in each case and, the symbols, the most probable values. The horizontal bar of length 1 d, centred on 25.3 February indicates the time interval in which the burst occurred. The variations of the length of each feature are also shown in arc degrees projected on the sky background.



their form over such a long period while travelling in interplanetary space. We noticed, furthermore, that the striae tend to lengthen with time. Figure 3 illustrates this point by showing the average length of the three striae as a function of time.

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Temperature dependence of flatband potentials at semiconductor-electrolyte interfaces

THE possible utilisation of photoelectrolysis for solar energy conversion suggests that the effects of temperature on such cells is important. One can envisage these cells being used in solar concentrator systems with a flowing electrolyte cooler. Thus operation at temperatures above ambient is possible and may offer significant advantages. The reaction rates on the surface of the semiconductor will be enhanced at the higher temperatures and there may be improvements in wavelength response and bias requirements of the cell. Thermal effects on the wavelength response of SnO_2 electrodes have been observed and attributed to thermal shifts in the bandgap¹. Here we explore the effects of temperature on the flatband potential V_{fb} and hence the bias requirements of a photoelectrolysis cell.

The experiments were carried out on single crystal TiO_2 electrodes cut perpendicular to the C-axis. The electrodes were reduced in a hydrogen atmosphere (700–800 °C) and had bulk resistivities of $0.3 \Omega \text{ cm}^{-1}$. A conventional three-probe electrochemical cell was used with a saturated calomel reference electrode and an electrolyte of 1M NaOH. The flatband potential was determined by measuring the photocurrent as a function of potential with the electrode illuminated with monochromatic light ($\lambda = 3,500 \text{ \AA}$). A plot of the square of the photocurrent gives an intercept at the flatband potential² as shown in Fig. 1. The temperature of the quartz electrochemical cell was controlled to within $\pm 1^\circ \text{C}$ using two heaters. Evaporation was limited by the use of a plexiglass cover over the cell. Averaging several runs such as those illustrated in Fig. 1, we find a shift $V_{fb}(20^\circ \text{C}) - V_{fb}(90^\circ \text{C})$ of $-0.03(0.01) \text{ V}$ (the number in parentheses is one standard deviation).

Several factors are important in determining the size of this shift in flatband potential. The flatband potential may be expressed as³:

$$eV_{fb} = E_A - E_o - e\Delta_{fc} - e\Delta_{pH} \quad (1)$$

where e is the electron charge, E_A is the electron affinity of the semiconductor, E_o is the position of the reference electrode relative to the vacuum level, Δ_{fc} is the potential difference between the bottom of the conduction band and the Fermi level and Δ_{pH} is the potential across the Helmholtz layer due to

adsorbed ions. All the terms on the right side of the equation are temperature dependent. Thus we expect that:

$$e[V_{fb}(20^\circ \text{C}) - V_{fb}(90^\circ \text{C})] = [E_A(20^\circ \text{C}) - E_A(90^\circ \text{C})] + [E_o(90^\circ \text{C}) - E_o(20^\circ \text{C})] + e[\Delta_{fc}(90^\circ \text{C}) - \Delta_{fc}(20^\circ \text{C})] + e[\Delta_{pH}(90^\circ \text{C}) - \Delta_{pH}(20^\circ \text{C})] \quad (2)$$

The temperature dependence of the electron affinity has not been measured or explicitly calculated for any compound. We have measured the variation with temperature of the bandgap of TiO_2 and find a decrease of $\sim 0.01 \text{ eV}$ between 20°C and 90°C . If this is due to shifts in both the conduction and valence band edges, we would estimate that the change in electron affinity is approximately -0.005 eV . The temperature dependence of E_o is due to the temperature dependence of the reference electrode and the concentration of the saturated KCl solution⁴. This shift is -0.05 eV . Another temperature-dependent factor is the position of the Fermi level in the semiconductor. From knowing the Seebeck coefficient as a function of temperature⁵ and the expression for the Seebeck coefficient, we can estimate the changes in Δ_{fc} . Neglecting minority carrier conduction, the Seebeck coefficient is given⁶ by

$$S = 86 [(e\Delta_{fc}/kT) + A] \quad (3)$$

where S is in $\mu\text{V K}^{-1}$, k is Boltzmann's constant, T , the absolute temperature, and A , a constant. Differentiating, we find

$$\frac{\partial S}{\partial T} = 86 \left[\frac{\partial \Delta_{fc}}{\partial T} \left(\frac{e}{kT} \right) - \frac{e\Delta_{fc}}{kT^2} \right] \quad (4)$$

The shift in Fermi level with temperature is then approximately

$$\delta \Delta_{fc} = \frac{\partial \Delta_{fc}}{\partial T} \Delta T = \left[\frac{\partial S}{\partial T} \left(\frac{kT}{86e} \right) + \frac{\Delta_{fc}}{T} \right] \quad (5)$$

Using Seager's data⁵, we estimate for TiO_2 that $\Delta_{fc}(90^\circ \text{C}) - \Delta_{fc}(20^\circ \text{C}) = 0.01 \text{ V}$.

The final temperature dependence comes for variations in the potential across the Helmholtz layer. This potential drop may be expressed³ as

$$\Delta_{pH} = 2.303RT(pH_{PZZP} - pH) \quad (6)$$

where R is the gas constant, T is the absolute temperature, pH_{PZZP} is the solution pH at which the number of adsorbed OH^- and H^+ ions is equal (point of zero zeta potential, PZZP) and pH refers to the electrolyte. The pH of a 1M solution of NaOH is temperature dependent because of the temperature dependence of the ionisation constant of water⁴. At 20°C the $pH = 14.1$ and at 90°C the $pH = 12.4$. The PZZP is also temperature dependent⁷: our measurements for TiO_2 give $pH_{PZZP}(20^\circ \text{C}) = 5.8$ and $pH_{PZZP}(90^\circ \text{C}) = 5.2$.

Thus we find that $\Delta_{pH}(90^\circ \text{C}) - \Delta_{pH}(20^\circ \text{C}) = +0.03 \text{ V}$. Summing the terms on the right-hand side of equation (2) predicts a shift in flatband potential of -0.035 V in excellent agreement with the measured value.

The bias required to operate a photoelectrolysis cell does not depend on the flatband potential alone but on the difference between it and the hydrogen evolution potential. The hydrogen evolution potential will also shift with temperature due to changes in solution pH and the Nernst slope. For our case this shift amounts to -0.07 V . Therefore, after removing the effects of the saturated calomel reference electrode from the flatband potential, the bias requirement changes for our cell on going from 20°C to 90°C is an increase in bias of 0.05 V . This is a relatively small change and suggests that if other considerations require operation at elevated temperatures, then the bias will not be a significant problem.

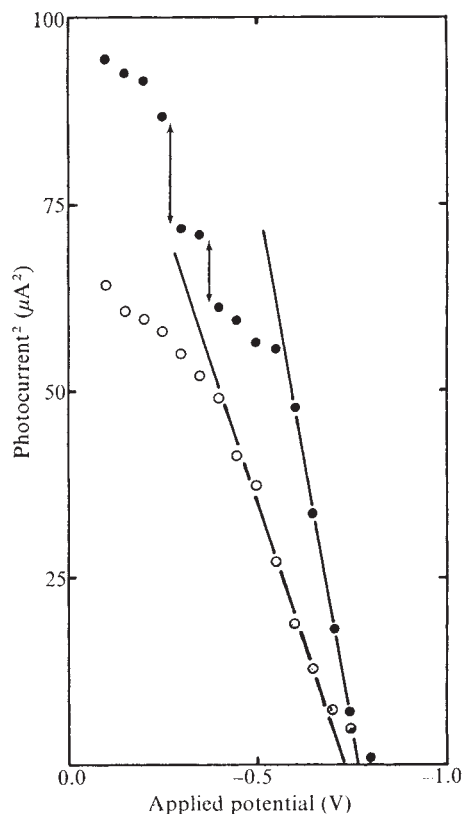


Fig. 1 The square of the photocurrent plotted against the applied potential for TiO_2 . The incident light is monochromatic with a wavelength of $3,500\text{\AA}$. The arrows indicate jumps in the lamp intensity. The difference in intercept for the two temperatures is about 0.04V . ●, 20°C ; ○, 90°C .

Are the results for TiO_2 in 1M NaOH typical of metal oxide photoanodes? The only factors which depend specifically on the semiconductor are the Fermi level shift, the electron affinity shift, and the PZZP shift. The Fermi level shift and electron affinity shift observed for TiO_2 are probably typical of highly doped metal oxide semiconductors and in any case would never be large enough to be significant. The behaviour of the PZZP has been explored by Berube and DeBruyn⁷. They show that for metal oxide electrodes:

$$4.606RT \left[\text{pH}_{\text{PZZP}}(T) - \frac{1}{2} \text{pK}_w(T) \right] = \Delta H - T\Delta S \quad (7)$$

where all the temperature dependencies are explicitly shown, K_w is the ionisation constant of water, ΔH is the difference in enthalpy required to transfer H^+ and OH^- ions from the solution to the semiconductor surface, and ΔS is the corresponding entropy difference. In general, if the potential determining species are H^+ and OH^- , then in an aqueous solution $T\Delta S$ will be quite small with respect to ΔH . As this is the case for TiO_2 (ref. 7), we need only examine the left side of equation (7) at the two temperatures of interest. Taking the typical extremes for an aqueous solution of 0°C and 100°C and a hypothetical PZZP at 0°C located at $\text{pH} = 14$, the maximum shift in the PZZP as calculated from equation (7) is about 3pH units. This corresponds to a potential shift of only 0.2V and shifts of less than this are expected for all oxides investigated so far. Thus the results for TiO_2 are representative of all metal oxide semiconductors used in photoelectrolysis cells. Similar arguments can be made for wet photovoltaic cells to account for the temperature dependence of their maximum open circuit voltages.

However, for potential-determining ions other than H^+ and OH^- the entropy contribution in equation (7) must be considered.

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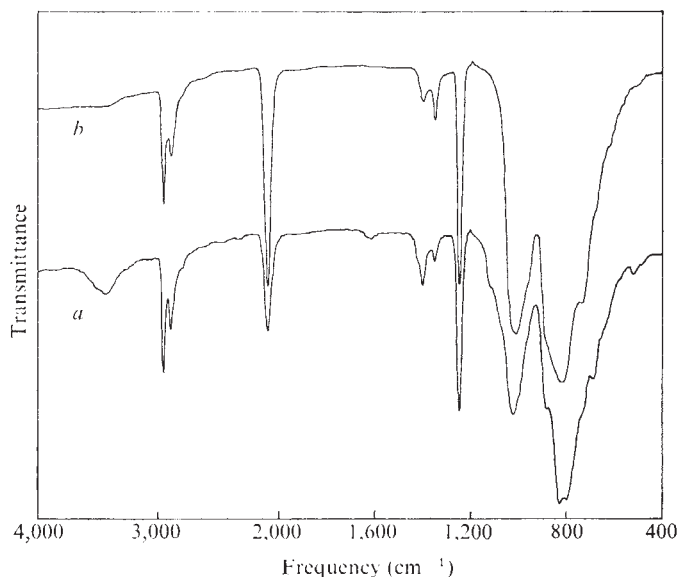
Development of high tensile strength silicon carbide fibre using an organosilicon polymer precursor

THE high tensile strength SiC fibre developed in our laboratory¹⁻⁵ is extremely heat-resistant and its wettability by metals is good. Metal-matrix composites reinforced with the SiC fibre should therefore be of practical use. Polycarbosilane, the precursor of the fibre, is synthesised by thermal decomposition under high pressure of polydimethylsilane in an autoclave. To produce the SiC fibre on an industrial scale, polycarbosilane needs to be produced in large quantities with high yield. The autoclave method requires a large amount of space, so the operation is inconvenient and uneconomic. We have, therefore, developed a new method of synthesising the polycarbosilane at normal pressure by adding several wt% of polyborodiphenylsiloxane⁶ to polydimethylsilane. The structure and properties of the new polycarbosilane, and the SiC fibre obtained from it are described here.

The new polycarbosilane was synthesised as follows. Polydimethylsilane, $(\text{Me}_2\text{Si})_n$, as the starting material was synthesised by dechlorination with sodium metal of dimethyldichlorosilane in xylene under N_2 gas⁷. Then, polyborodiphenylsiloxane $(\text{Ph}_2\text{Si}-\text{O}-\text{B})_n$ was synthesised by first

heating boric acid, $\text{B}(\text{OH})_3$, and diphenyldichlorosilane, Ph_2SiCl_2 , in *n*-butyl ether at 100°C under N_2 gas and then

Fig. 1 IR spectra of (a) PC-1 and (b) PC-2.



further heated under reduced pressure to 400 °C; 250 g of the polydimethylsilane was mixed with 8.26 g of the polyborodiphenylsiloxane, (PB)_p. The mixture was put in a 2-litre quartz reaction vessel equipped with reflux condenser, it was then heated under N₂ gas in an electric furnace, after reaction for 6 h at 350 °C, it was cooled to room temperature. The resinous product was removed as a benzene solution, and after filtration, the benzene was removed. Then the components with boiling points up to 280 °C were removed by vacuum distillation at 1 torr. The product obtained was 125 g (yield 48.4%) of polycarbosilane, PC-1; its number average molecular weight was 1,860. Chemical analysis of the product showed 44.5 wt% Si, 35.8 wt% C, 4.83 wt% O and 8.00 wt% H; and spectrochemical analysis showed several 10 p.p.m. B. The empirical formula was SiC_{1.88}O_{0.19}H_{5.03}. For comparison, 147 g (yield 58.8%) of polycarbosilane, PC-2, with average molecular weight 1,750, was also synthesised by heating, in an autoclave, 250 g of polydimethylsilane at 470 °C for 14 h. Its empirical formula was found by chemical analysis to be SiC_{1.77}O_{0.03}H_{3.70}.

Figure 1 shows the IR spectra of PC-1 and PC-2. In both curves absorptions at 1,020 and 1,355 cm⁻¹ (Si-CH₂-Si) and 2,100 cm⁻¹ (Si-H) (refs 8, 10), indicate the formation of polycarbosilane. The ¹H-NMR spectrum of PC-1 reveals the presence of phenyl groups. From the UV absorption spectrum

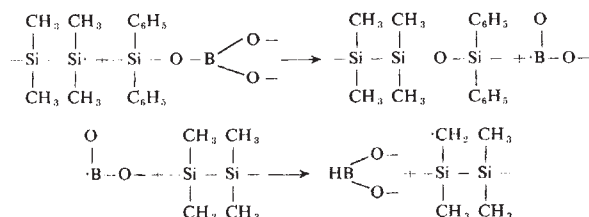
of PC-1, small quantities of $-(Si)_2-$ are seen to be contained in

the molecules. Figure 2 shows DTA curves of PC-1 and PC-2, heated from room temperature to 500 °C, at 5 °C per min in air. The exothermic peaks at lower temperatures represent oxidation by O₂ with weight gain. The peak in PC-1 is at lower temperature by about 120 °C than that in PC-2. This indicates a difference in molecular structure between PC-1 and PC-2. This agrees with the fact that the heating temperature, in air, required to make the spun fibre infusible, is far lower in PC-1 than in PC-2.

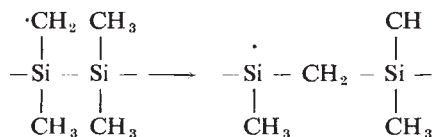
From the results of the chemical analysis, the following model of PC-1 formation mechanism can be postulated. In the initial stage of reaction, the main chain of polydimethylsilane is first decomposed locally, producing radicals. Increasing the temperature causes the polydimethylsilane to decompose into low molecular components and the concentration of the radical rises. If then (PB)_p exists, the B-O bond in it is severed through the action of the produced radical.

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TWO



As indicated above, the low molecular components resulting from thermal decomposition of polydimethylsilane are trapped and at the same time hydrogen is drawn out of the methyl group in polydimethylsilane. Therefore, formation of the carbosilane skeleton in radical transition reaction¹¹



is enhanced. Rise in temperature of the reaction system is thus not hampered by reflux of the low molecular components, and the thermal decomposition of polydimethylsilane and the formation of polycarbosilane by a radical transition reaction are attained. The severance of the B-O bond in (PB)_p is confirmed

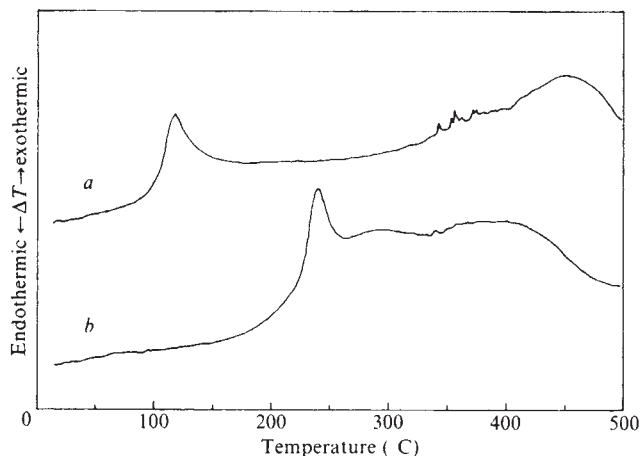


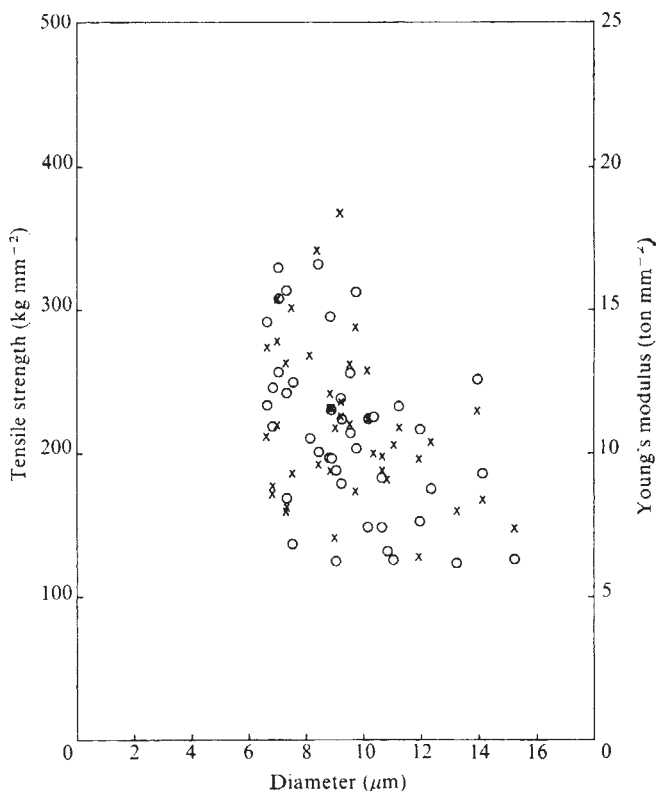
Fig. 2 DTA curves of (a) PC-1 and (b) PC-2.

by chemical analysis, showing that in PC-1 there exists O and scarcely any B, and B is released in the form of gaseous products from the system.

The skeleton of PC-1 synthesised by this new method is principally -Si-C- but also contains $-(Si)_2-$ and $-Ph_2Si-O-$, so

the molecular structure is more linear than PC-2 and, therefore, PC-1 will have high spinnability. Furthermore, the strength of the spun fibre is higher than that of the spun fibre of PC-2. Once made infusible, the strength is especially high, 2–5 kg mm⁻² (the strength in PC-2 cannot be measured) so that handling the fibre is very easy. Raising the temperature under N₂ gas to 1,300 °C, at 5 °C per min, the weight residue in PC-1 is 67.8%, which is significantly larger than the 54.5% found with PC-2. This is also advantageous in obtaining the SiC fibre. Figure 3 shows the relationship between fibre diameter and tensile strength and between fibre diameter and Young's modulus in the SiC fibre obtained by melt-spinning PC-1 at 310 °C, making it

Fig. 3 Tensile strength (○) and Young's modulus (×) of SiC fibre.



infusible by heating in air to 120°C and finally in vacuum to 1,300 °C. Like the conventional fibre, it is a uniform cohesion of fine β -SiC particles, the crystal structure having no orientation. Results in Fig. 3 seem to be somewhat inferior to those in the conventional SiC fibre^{1,3,4}. However, the mechanical properties will no doubt be improved by optimising the conditions of variables as PC-1 synthesis, making them infusible and firing. The new synthesis method using polycarbosilane as the precursor for SiC fibre should facilitate mass production of continuous silicon carbide fibre.

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Constitution and significance of the Troodos sheeted complex

THE Troodos ophiolitic suite (Cyprus) has long been considered as a model of a piece of oceanic crust¹⁻⁴ with its sheeted complex as the proof of accretion during Mesozoic times^{5,6}. Using geochemical data, however, it has been

suggested⁷ that the Troodos may have been formed in an island arc environment. Thus, a discussion has arisen on the origin of this ophiolite⁸⁻¹⁰. New fieldwork and detailed studies show that the Troodos sheeted complex consists of two main structurally well defined families of dykes, the first one partly produced by accretion, the second related to accretion and late fracturing of a magma chamber when spreading stopped. All the dykes are tholeiitic and four generations have been recognised: diabase, dolerite, picritic lamprophyre and basalt—this succession may be compared to the evolution of the Troodos pillow lavas¹¹. New geochronological measurements reported here suggest that the emplacement of the sheeted complex started before 80 Myr, and confirm the existence of an active 'tethyan' ridge in middle cretaceous. Isochrone ageing show that greenschist facies metamorphism is also pre-Campanian.

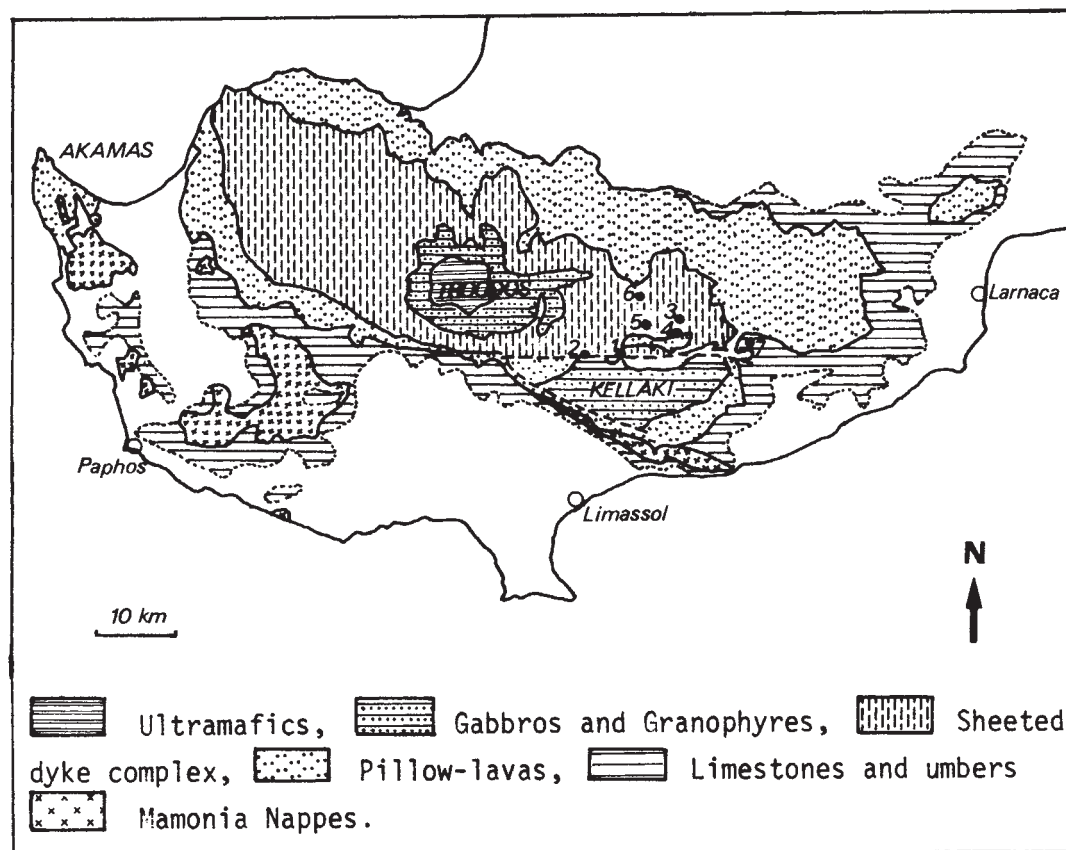
The sheeted complex of the Troodos is one of the most developed of all the Eastern Mediterranean ophiolites. It consists of thousands of dykes exposed over a very large area; the host rocks of plutonic character are subordinate and do not represent more than 2-5% of the complex.

The main strike of the dykes is north-south, but, from west to east, it progressively changes from N 160° E to N 50° E and even to N 90° E (ref. 12) along the Arakapas-Athrakos fault zone, presently considered as a palaeo-transform fault¹³.

Fieldwork has been carried out in the eastern Troodos, in the Odhou-Pharmakas-Lefkara area (Fig. 1) where metamorphism and hydrothermal alterations are less developed than in the Western areas.

Four generations of dykes have been recognised (Fig. 2). (1) The oldest ones (from 0.5 to 0.8 m thickness) consist of diabases some of them with greenschist paragenesis and show asymmetrical chilled margins (type 2). They intrude into a coarse grained dolerite (type 1) without any chilled margin, considered as the roof of the underlying magma chamber. They are frequently parallel, but may be divided into two crossing sub-generations, the earlier more or less

Fig. 1 Map of the Troodos Massif and the Pap-hos area (Cyprus). 1, Arakapas; 2, Athrakos; 3, Ayi Vavatsinia; 4, Ora; 5, Odhou; 6, Pharmakas; 7, Lefkara.



horizontal, the later vertical or high dipping.

(2) Generally the N 65° E trending doleritic dykes with conjugated high dips (type 3) are more developed and numerous. They are 1.8–2.5 m wide, show symmetrical chilled margins, prismatic columnar joints and are mostly parallel to the second sub-generation of type 2.

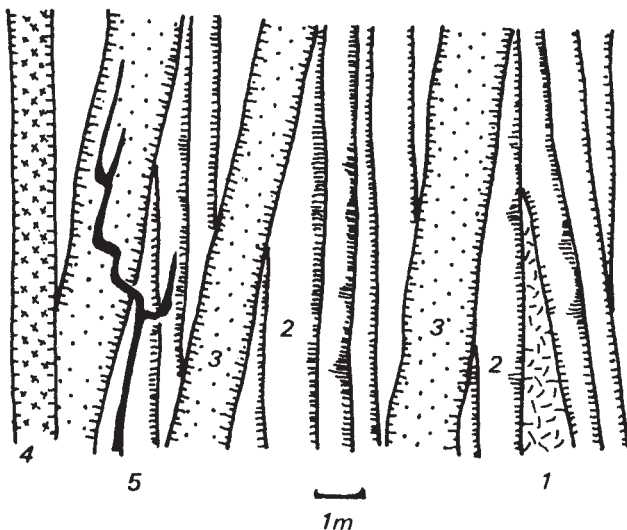
(3) Less abundant dykes of very basic composition have also been discovered. They range from 0.8 to 2.0 m wide and present regular symmetrical chilled margins and abundant ultramafic phenocrysts in the middle of the dyke, giving to these rocks a 'lamprophyric' or picritic aspect (type 4).

(4) Finally, some small (0.10–0.50 m) and sinuous, sometimes bigger (up to 1.5 or 2.0 m wide), volcanic dykes are observed running along joints or intruding into diabasic and doleritic dykes.

In many north-south sections of the Troodos sheeted complex, especially along the road from Melini to Layia, west of Ora village, near the Arakapas-Athrakos Fault Zone which separate the Kellaki Massif (Limassol forest area) from the Troodos, two sub-orthogonally cutting families of dykes have been discovered. The first ones, consisting of diabasic N 135° E trending dykes (type 2) intrude into granophyres and coarse grained dolerites. The second ones, which trend N 60° E, cross the former and consist of doleritic dykes of type 3, parallel to or cut by some lamprophyric or basaltic dykes (type 4 and 5).

In spite of their scarcity, three kinds of these host-rocks have been recognised: layered gabbro, doleritic gabbro and granophyre. From our observations, the coarse grained dolerite (type 1) which locally grades to diorite or to amphibole granophyre, must be added, being probably the most abundant host-rock of the Troodos sheeted complex. The layered gabbros are rare but consist of basic plagioclase (An 70–75) and green, probably secondary, amphibole. The doleritic gabbros are more abundant and consist of augite, altered plagioclase, some orthopyroxene and quartz in pegmatitic association with feldspar. The granophyres (trondhjemites or plagiogranites) outcrop as veinlets intruding into the gabbros or as screens between the dykes. They show doleritic textures with laths of plagioclase (An 35–50) surrounded by green hornblende and epidote, and frequently contain micropegmatite. In the most evolved or superficial (dacites) granophyres, albite and quartz phenocrysts with epidote are observed. The coarse-grained dolerite consists of zoned plagioclase, actinolite and hornblende with, some-

Fig. 2 Synthetic section showing the successive dykes families: 1, host-rocks (coarse grained dolerites); 2, five grained diabasic dykes with asymmetrical chilled-margins; 3, doleritic dykes; 4, basic dykes (picritic); 5, metabasaltic dykes. 3, 4 and 5 have regular symmetrical chilled-margins.



times, pegmatitic association of quartz and plagioclase. This suggests that the quartz is primary. Indeed, in some metasomatically transformed facies, secondary quartz may be observed.

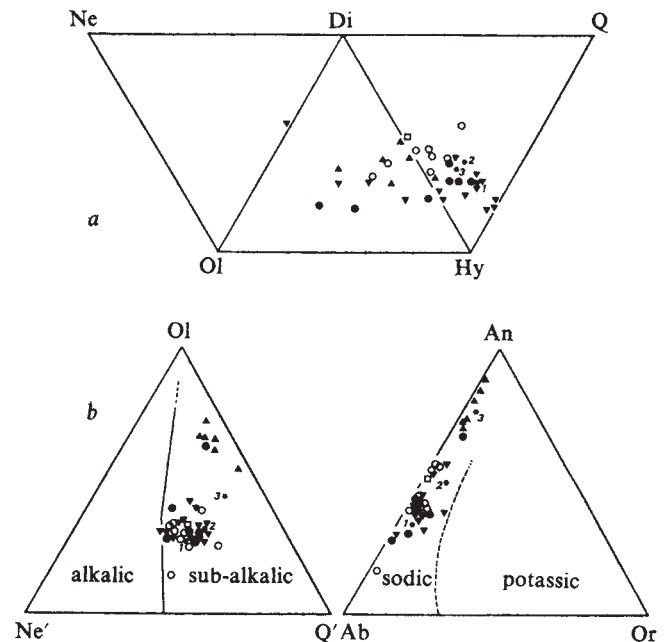


Fig. 3 a, Q-Hy-Di-Ol-Ne diagram from Yoder and Tilley (1962); b, Ol-Ne'-Q' and An-Ab-Or diagrams from Irvine and Baragar (1971) illustrating the tholeiitic character of the dyke complex of the Troodos Massif, Cyprus. □, facies 1, doleritic host-rocks; ○, facies 2, fine-grained diabasic dykes; ▲, facies 3, doleritic dykes; ▲, facies 4, basic dykes with picritic affinities; ●, facies 5, metabasaltic dykes. * 1, 2 and 3, average values of BG, LPL and UPL (ref. 11).

The dykes of the first family (type 2) principally consist of diabasic dykes which always present only one chilled margin. They are often parallel but sometimes intersect with an angle between 30 and 50°. Although relatively abundant near the core of the Troodos, they are present everywhere in various amounts. Most of them have porphyritic or vesicular intersertal texture with phenocrysts of plagioclase and ouralised clinopyroxene in a fine-grained matrix made of needles of albitised plagioclase embedded in a chlorite-epidote-quartz assemblage. In the most transformed samples (albite, quartz, actinolite and chlorite) of the greenschist assemblage, the primary magmatic textures have completely vanished. In contrast, some less transformed dykes show fresh clinopyroxene and partly albitised plagioclase.

The three last generations of dykes (types 3, 4 and 5) can be grouped into a second family because they all present two symmetrical chilled margins. They mostly intrude into the above-mentioned diabasic dykes (first family) in a more or less parallel direction, but they can sometimes cross at an acute or wide angle (for instance, in Ora or in Ayii Vavatsinia, Fig. 1).

The type 3 mineralogy is typically doleritic with fresh augite sometimes in phenocrysts, plagioclase (An 35–40) slightly altered with albite rims, chlorite and quartz. These dykes are the most abundant sheeted complex (up to 70%) and are present everywhere. They are very often unaltered.

The basic dykes, with lamprophyric or picritic affinities (type 4) have a very basic composition and the high content of mafic minerals (up to 70%) give them a picritic character. Many of them were found near the Arakapas-Athrakos fault, but they also outcrop in all the external zones of the sheeted complex. The rock shows cumulative texture with abundant and large (up to 3 and 4 cm) altered olivine and orthopyroxene, fresh clinopyroxene phenocrysts cemented by basic plagioclase (An 50–70), smaller and fresh

Table 1 K/Ar age determinations

Sample no.	Type	% K	^{40}Ar rad ($\text{mol g}^{-1} \times 10^{-10}$)	$^{40}\text{Ar}/^{36}\text{Ar}$ ($\times 10^3$)	$^{40}\text{Ar}/^{36}\text{Ar}$ ($\times 10^5$)	Age (Myr)
PK 2	2	0.30	0.423	0.903	1.30	78.6 $\pm$ 4.3
PK 60a	2	0.15	0.191	0.547	0.599	71.0 $\pm$ 5.8
PK 60b	2	0.15	0.239	0.689	0.727	91.0 $\pm$ 6.3
PK 57	2	0.18	0.259	0.522	0.468	81.6 $\pm$ 6.3
PK 13	4	0.05	0.138	0.348	0.0583	150.0 $\pm$ 9.0
PK 13	4	0.07	0.189	0.373	0.0811	158.1 $\pm$ 9.2
PK 67	1	0.13	0.285	0.394	0.134	122.4 $\pm$ 22.2
PK 52b	3	0.45	0.794	0.831	0.906	99.0 $\pm$ 5.8
CY 92	3	0.45	0.204	0.541	0.506	81.6 $\pm$ 7.5
PK 81b	5	0.28	0.544	0.444	0.233	106.8 $\pm$ 11.5
PK 4	4	0.04	0.111	0.337	0.0411	165.9 $\pm$ 14.8
PK 59	4	0.03	0.0565	0.343	0.0806	99.1 $\pm$ 21.8

$$\lambda^{40}\text{K}\beta^- = 4.962 \times 10^{-10} \text{ yr}^{-1}$$

$$\lambda + \lambda^{40}\text{K}^e = 0.581 \times 10^{-10} \text{ yr}^{-1}$$

olivine crystals (0.3–0.5 cm), ortho and clinopyroxenes and a few secondary minerals, such as quartz, chlorite, serpentine and actinolite.

The olivine metabasalts (type 5) are tiny, sinuous dykes with basaltic composition of altered olivine and quite fresh clinopyroxene phenocrysts. Close to the chilled-margins, the lava shows fluidal texture with plagioclase microlites (An 55) parallel to the dyke edges. Though discrete near the gabbros, they are present in all parts of the complex and become more numerous towards the pillow lavas series.

Our discussion is supported by Desmet *et al.*'s¹⁴ analysis of 45 new major elements and our interpretation by the classical diagrams of Irvine and Baragar¹⁵, and Yoder and Tilley¹⁶. This should be confirmed by trace elements analyses, because most of the rocks of the sheeted complex are partly altered.

The O1' Ne' Q' and An Ab Or diagrams (Fig. 3b) show that all the plotted samples belong to single magmatic unit. Although type 4 seems to be a little different, due to the abundance of mafics, types 1, 2, 3 and 5 are strictly cogenetic.

On the Q-Hy-Di-O1-Ne diagram (Fig. 3a), types 1, 2, 3 and 5 show the same trend, from quartz tholeiite to olivine tholeiite and we notice the good correlation between the dolerites 3 and metabasalts 5. The picritic dykes of type 4 cover the olivine tholeiite field.

Albitisation of the diabasic dykes (type 2) is probably due to secondary phenomena which can be easily observed in the Ab-An-Or diagram. The pillow lavas of the basal group, with some greenschist facies paragenesis⁵ have probably been fed by such dykes. Types 3 and 5 have the same petrographical and geochemical compositions and the average values of the lower and upper pillow lavas, plotted

on Fig. 3 show that they can be considered as their feeder dykes¹¹.

Finally, the picritic or lamprophyric dykes (type 4), with their very basic composition, can be related to some ultrabasic flows, the so called 'ultrabasic pillow lavas', which appear locally in the upper pillow lavas¹¹.

The samples K/Ar were dated (by M.D.); potassium was measured by flame photometry and argon by isotope dilution techniques with an AEI MS-10-S mass spectrometer. The samples were prebaked overnight at 180 °C. The results are given in Table 1.

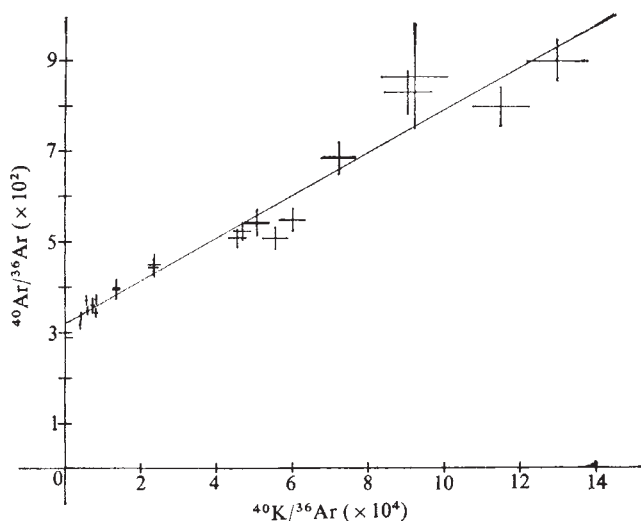
The ages show a large spread between 70 and 165 Myr. These K/Ar ages are not consistent with the cross-cutting relationship displayed by the dykes. If the $^{40}\text{Ar}/^{36}\text{Ar}$ data are plotted against $^{40}\text{K}/^{36}\text{Ar}$ isotope ratio we obtain a good isochrone (correlation factor of 0.9756), with an age of 79.2 ± 3.8 Myr and with a $^{40}\text{Ar}/^{36}\text{Ar}$ intercept of 319 ± 12 (errors quoted at the 95% confidence level). This ratio is significantly higher than the atmospheric argon ratio of 295.5, indicating the possibility of a small argon overpressure (Fig. 4).

Vine *et al.*¹⁷ considered the dykes to be pre-Campanian in age on the basis of the radiolarian assemblages in the sediments overlying the pillow lavas. Among their four published ages, only two are pre-Campanian and the two others are not interpreted. Our isochrone age, based on 12 determinations (many of them are the mean of duplicate runs) is a minimum age of the extrusion of the dykes. This age is not contradicted by the younger palaeontological age of the overlying sediments. The age of 79 Myr (Santonian–Upper Cretaceous) should probably be related to the greenschist facies metamorphism and the real age of dykes emplacement probably earlier (maybe in Albian–Cenomanian times).

The alteration of the rocks (probably due to the metamorphism) is variable from sample to sample. It is worth noting that the less transformed rocks yield the oldest ages; however, these samples have no $^{40}\text{K}/^{36}\text{Ar}$ ratios so that they cannot be individualised on a particular isochrone showing an older age. We need a true isochrone mid-Cretaceous age, or proof that no argon overpressure exists in these samples so that the calculated age is really the formation age.

These results indicate that the sheeted complex of the Troodos ophiolitic suite is well organised, and the structural and petrological evolution of the different families of dykes proves it. Most of the type 2 dykes with asymmetrical chilled margins may result from an accretion process along a Mesozoic mid-tethyan ridge. But some, which are locally parallel to the banding of the gabbros, may be regarded as primary sub-horizontal units, like sills, produced by progressive cooling at the top of the magma chamber. The same material may intrude the roof of the magma chamber and give rise to the first generation of dykes when spreading starts. These diabasic dykes or sill-like bodies intrude into doleritic gabbros, granophyres and coarse-grained dolerite (type 1) which may be regarded as

Fig. 4 K/Ar isochrone ageing on Troodos samples.



the more internal components of the roof of the magma chamber. The second types (3, 4 and 5), characterised by symmetrical chilled margins, are more complicated. The vertical doleritic dykes of type 3, intersected by lamprophyric (picritic) and basaltic dykes of types 4 and 5, are the most numerous of the complex. Their abundance may represent a large volume of spreading, in spite of regular symmetrical chilled margins, if we consider an accretion model⁶ in which the oceanic spreading only produces dykes with asymmetrical chilled margins; this implies that the spreading axis is always unique and constant, but our observations suggest that the spreading may be produced, in some cases, by an accretion zone (and not by a single axis) in which all the dykes may intrude into each others.

The dykes of types 4 and 5, of volcanic affinities, may represent the last products of the magma chamber emplaced after roof fracturation (with normal faulting). Thus, they probably represent a third stage in the evolution of the Troodos ophiolitic complex, after the magma chamber formation with its crust, and the spreading.

Nevertheless, we do not know much about the nature and the condition of the roof in the magma chamber before and during the emplacement of dykes. We are not sure whether the type 3 dykes belong to an expanding system or to simple fracturation. Moreover, studies now in progress may determine the true modalities of their emplacement, the dynamics of the fracturation or of the opening, and confirm this model. No sheeted complex like the troodos has been previously observed in a mid-oceanic ridge environment.

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Soil as a source or sink for atmospheric nitrous oxide

INCREASED use of nitrogenous fertiliser has been thought to cause¹⁻⁴ greater nitrous oxide (N_2O) production by soils and natural waters. After transport to the stratosphere, this additional N_2O may be converted to nitric oxide (NO) and

deplete the ozone layer⁵. However, there is a diversity of opinion about the probable effects of increasing nitrogenous fertiliser use on the ozone layer⁶⁻⁸. The controversy cannot be settled until there is reliable information on (1) the rate of N_2O production from both natural sources and fertilised soils; (2) the concentration of N_2O in the atmosphere; and (3) the existence and capacity of sinks for N_2O at the Earth's surface or in the troposphere^{9,10}. Brice *et al.*¹¹ have reported evidence for an appreciable but unidentified ground surface sink. We report here the results of investigations designed to identify the soil moisture conditions for which soil could act as that sink. We found, in both the laboratory and the field, that at soil moisture contents less than field capacity, N_2O was emitted from soils; there was no evidence that soils in the field ever absorbed N_2O . In laboratory studies some N_2O was absorbed (as reported elsewhere¹²), but the phenomenon was transitory, probably resulting from a low oxygen content in the atmosphere above the soil. Nitrous oxide was eventually emitted again when the atmosphere was returned to normal. These results suggest that, in the field, soils are more likely to be sources than sinks of N_2O for the atmosphere.

Laboratory measurements were made in duplicate on soil

Fig. 1 Absorption and emission of N_2O by waterlogged soils. Soils (30 g) were covered with water to a depth of 1 cm in 150-ml conical flasks. Flasks were flushed with ambient air at times indicated by arrows. Other experimental conditions were as described in Table 1. Note the break in the scale; points below the break represent zero values. Before the flasks were flushed with ambient air the oxygen concentration above soils 4, 6 and 7 were 7, 0.2 and 10% respectively. Δ , Soil 4; $\blacksquare$, soil 6; $\circ$, soil 7; p.p.b., parts per 10⁹.

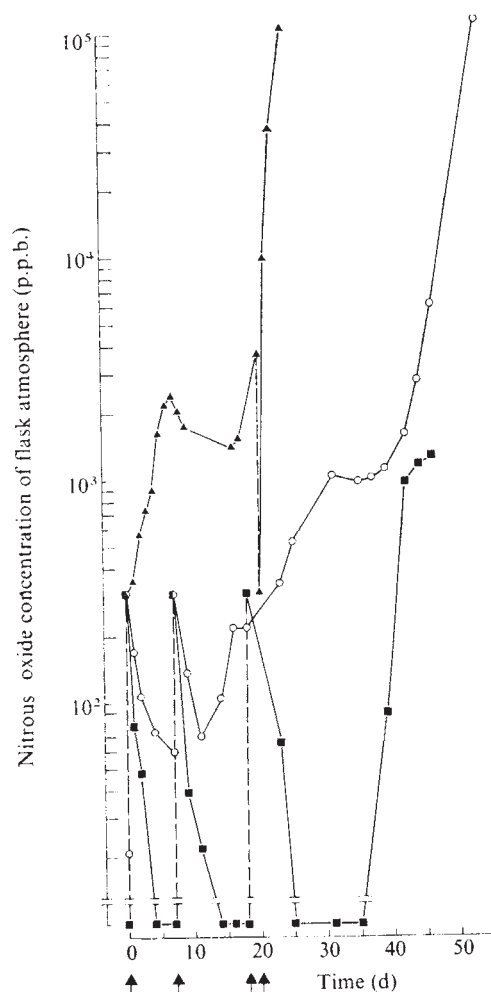


Table 1 Nitrous oxide emission by soils at low moisture contents

Soil	% of saturation	Total N %	NH ₄ -N (µg N per g soil)	NO ₃ -N (µg N per g soil)	Time after addition of water (min)									
					0	6	12	18	24	30	36	42	54	60
					Nitrous oxide produced (µg N per kg soil)									
1	4	0.46	18	320	0	0.05	0.13	0.21	0.31	0.40	0.53	0.60	0.87	0.98
2	13	0.21	31	186	0	0.07	0.09	0.13	0.13	0.16	0.20	0.24	0.31	0.33
3	14	0.10	8	11	0	0.07	0.09	0.11	0.19	0.16	0.19	0.20	0.33	0.35
4	15	0.09	10	15	0	0.01	0.03	0.05	0.09	0.11	0.12	0.15	0.17	0.19
5	8	0.09	5	9	0	0.04	0.09	0.13	0.17	0.21	0.32	0.32	0.49	0.50
6	13	0.39	6	6	0	0.07	0.09	0.12	0.14	0.15	0.20	0.22	0.29	0.33
7	15	0.09	73	2	0	0.01	0.01	0.03	0.06	0.07	0.08	0.08	0.10	0.11
8	20	0.11	3	4	0	0.05	0.06	0.07	0.11	0.13	0.08	0.08	0.15	0.16

A sample of 30 g soil was incubated with the appropriate amount of water under a normal air atmosphere, in 150-ml conical flasks stoppered with rubber septa, at 25 °C. Gas samples were withdrawn by a gas-tight syringe after the atmosphere in the flask had been mixed by pumping the plunger of the syringe sharply 12 times. N₂O was determined by gas chromatography using an electron capture detector¹³. The gases were separated on 2.5 m × 3 mm (o.d.) columns of Porapak Q and Porapak R (ref. 14), with argon-5% methane carrier gas at 20 ml min⁻¹, an oven temperature of 35 °C and a detector temperature of 350 °C. A standard curve was prepared by diluting pure N₂O with pure N₂ in calibrated flasks. In calculating results, allowance was made for the solubility of N₂O in water. A standard sample of air was analysed on each sampling occasion so that corrections could be made for any instrumental fluctuations. Suitable controls were included with each experiment to determine N₂O gain or loss from or through rubber septa, water and so on. When studying the effect of water addition, dry soil controls were included. The soils studied ranged in pH from 5.0 to 7.1 and in texture from a fine sandy loam to a light clay.

samples in 150-ml conical flasks. The appropriate amount of water was distributed from a pipette to simulate natural rainfall, but the flasks were not shaken to mix the wetted soil. Other methods of adding water were compared and the method of water addition had little or no effect on the evolution of N₂O.

Field measurements on undisturbed soil were made on a mown grass-clover sward, 50 × 50 m in area, adjacent to the laboratory in Canberra. The sward was unfertilised. Foliage was removed from an area of 4 m² by clipping and a specially constructed emission chamber enclosing a soil area of 700 cm² was installed in its centre. The N₂O enrichment of air drawn through the chamber was measured with an infrared gas analyser fitted with 30-cm measurement cells and operated in differential mode. Enrichments as small as 6 p.p.b. (parts per 10⁹) could be measured accurately. The rate of N₂O emission was calculated from

$$F = \rho \delta v / A$$

where F is the flux density of N₂O; ρ , the density of N₂O; v , the volume air flow rate through the chamber; A , the surface area; and δ , the difference in N₂O concentration between the air in the chamber and ambient air.

Gases which could interfere with the determination (water vapour, CO₂, CO, NO and NO₂) were first removed. After scrubbing, the combined effect of any interference from these constituents is less than 1 p.p.b. N₂O. Further details of this technique will be described elsewhere.

In short-term laboratory studies the N₂O content of the flask atmosphere was monitored at 6-min intervals (for 1 h) after adding very small amounts of water. Nitrous oxide was released from all soils studied immediately after water was added (Table 1). The initial rate of evolution of N₂O did not seem to be related to any single soil property or previous treatment of the soil. Both air-dried and field-moist soils released additional N₂O when water was added.

No N₂O was released from autoclaved soils or soil treated with formaldehyde to inactivate microorganisms. As there was no lag in production of N₂O (Table 1) there must have been an active microbial population or enzyme system present in these soils capable of producing N₂O as soon as water was added.

In longer-term laboratory studies on one soil the N₂O content of the flask atmosphere was monitored for 20 d after adding various amounts of water. The rates of N₂O production increased markedly with increased water additions (Table 2). At moisture contents less than field capacity the soil seems to act mainly as a source of N₂O. Air-dried soils also emitted N₂O but the rate of emission was very slow (Table 2).

The field study with undisturbed soil confirmed that, at moisture contents up to field capacity, soil acts as a source for N₂O. Nitrous oxide was emitted from dry soil and wet soil, but the rate of N₂O emission from the dry soil was much lower. Typical rates of N₂O emission for a 2-h period around noon were 0.06, 0.2 and 1.1 g N₂O-N per hectare per h, respectively, for a dry soil which had not received any rainfall or irrigation water for at least 6 weeks, for the same soil immediately after adding 5 mm of water, and again after adding 50 mm of water.

Field observations extending for several days at a time have been made at the same site, at irregular intervals, over a period of 5 months. The surface moisture content has ranged from air-dry to near saturation. On every occasion the soil has emitted N₂O. The field chamber is operated in such a way that the concentration of N₂O does not build up to levels much higher than normal atmospheric concentrations, and the oxygen content is not reduced appreciably below normal.

In the laboratory studies the oxygen content of the gaseous phase was found to decrease detectably only when

Table 2 Nitrous oxide* detected in the gas phase (and dissolved in water) for soil 4 incubated in air at different soil moisture levels at 25 °C

Water added (ml per 30 g soil)	Time (d)												
	0.3	1	2	4	6	8	10	12	14	16	18	20	Soil nitrate after 22 d (µg N per g soil)
Nitrous oxide (µg N per kg soil)													
0	0	0	0	0	0.3	0.4	0.5	0.6	0.7	0.7	0.8	0.8	15
2	0.9	2.7	5.4	8.9	15.1	20.0	23.8	27.0	29.0	28.3	28.8	28.7	37
4	1.2	3.1	5.9	9.6	14.2	16.9	18.0	21.4	26.3	23.3	23.8	24.3	40
6	1.5	35.3	112.3	153.0	176.5	181.1	185.8	194.6	186.4	196.5	191.9	188.4	49
8†	33.8	577.5	661.6	548.8	440.4	339.1	305.0	166.3	200.4	149.5	115.1	92.1	45
10	340.0	2464.6	1789.2	724.7	631.8	369.0	198.2	117.5	62.7	20.9	15.2	9.0	0
15	333.2	2942.6	1782.6	891.4	567.4	297.7	140.3	65.8	29.3	10.9	3.5	1.0	0
20	153.0	261.8	182.9	99.5	57.1	32.2	17.1	9.2	4.2	1.9	0.8	0.3	0

*Excess over starting conditions; more than 93% was present in the gas phase.

†Field capacity (100 cm tension); 13 ml of water are required to saturate the soil. Experimental details were as described in Table 1. Soil nitrate was determined concurrently on a replicate series of soils by extraction with 0.5M CaCl₂ solution and distillation¹⁵.

the soils were waterlogged. At moisture contents up to field capacity, the soils seemed to be aerobic overall, because of the high oxygen content of the atmosphere and because nitrate was being produced from the soil organic nitrogen and ammonium (Table 2). This reaction is known to require aerobic conditions¹⁶. These results suggest that soils which are essentially aerobic act as sources of N_2O . This probably applies to the whole range of soil moisture conditions from air-dry to field capacity.

We are uncertain about the mechanism of N_2O production in these experiments. The gas may have been produced in anaerobic microsites by denitrification of nitrate, but studies with inhibitors of the reaction suggest that this was not so. Azide, for example, which inhibits the reduction of nitrate¹⁷, did not stop the emission of N_2O from the soils at low water contents either in the laboratory study, or in the field. It is possible that N_2O was evolved during the oxidation of ammonia to nitrite. This reaction has been shown to occur in pure cultures of *Nitrosomonas europaea*^{18,19}.

In laboratory studies, at moisture contents equal to or greater than field capacity, the soil seemed to act as a source of N_2O for a short time and then as a sink; that is, the N_2O concentration in the gas phase increased and then decreased (Table 2). Presumably the N_2O was reduced to N_2 (ref. 12). At moisture contents above field capacity, nitrate was rapidly removed which agrees with earlier descriptions of denitrification.

In these laboratory experiments, however, we worked with a closed system and the N_2O concentration above the soil increased to a level far in excess of that possible in the field, where the normal circulation of the atmosphere would remove the N_2O soon after it was formed. Thus the rate of absorption of N_2O at high ambient N_2O concentrations cannot be realistic. An equally important difference from field conditions is that the oxygen concentration in the flask atmosphere was markedly lower than that occurring in the field.

Hence, when all of the soil nitrate had been metabolised and the N_2O concentration in the flask had been reduced to near zero, the air in the flask was replaced with normal air and the rate of reduction of N_2O at ambient concentrations was monitored. Comparison of Fig. 1 and Table 2 shows that the rate of N_2O absorption at low N_2O concentrations was much slower than that at high concentrations. Each time the concentration was reduced to or near zero, the cycle of flushing and monitoring was repeated. (Any moisture loss during flushing was replaced with distilled water.) The results show that after several cycles, the soil began to emit N_2O again. (Fig. 1).

These results are readily explained in terms of the nitrification-denitrification cycle outlined by Patrick and Reddy²⁰. When a waterlogged soil is in contact with an aerobic atmosphere an oxidised zone is formed in the top few millimetres of soil. Ammonium ions formed by decomposition of organic nitrogen diffuse to this oxidised zone where they are nitrified. Nitrate so formed diffuses back to the anaerobic zone and is denitrified.

Laboratory studies suggest that over the whole range of moisture conditions, from air-dry to saturated, only the very wet soils have any appreciable capacity to act as sinks for atmospheric N_2O . This can only happen in the field during prolonged periods in which the soil profile remains very wet; such conditions are unlikely to occur over a large part of the landscape. The more likely result of intermittent wetting by rain or irrigation would seem to be a net emission of N_2O from the soil to the atmosphere. Our results suggest that even very wet soils are more likely to be sources than sinks as long as the surface soil has an oxidised layer and mineralisable organic nitrogen is present.

Confirmation of these findings will require continuous

field observations made in a variety of moisture and temperature conditions with normal atmospheres.

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Reset Rb/Sr whole-rock systems and chemical control

THE susceptibility of fine-grained, volcanic rocks to open-system behaviour during regional metamorphic activity has long been suspected in Rb/Sr geochronology¹⁻⁵. The evidence that we present here shows that not only can open-system behaviour occur, but that it is partly a function of bulk chemical composition. We have studied the Burlington Peninsula area of northern Newfoundland (Fig. 1) which is underlain by the Cape St John Group, a volcanic pile of sub-aerial, calc-alkalic volcanic and sedimentary material, about 3,500 m thick. Coarse calcareous units and minor conglomeratic beds form its basal part; overlying units consist of basic volcanic flows and sills, commonly vesicular, associated with andesitic and silicic pyroclastic rocks and flows. The Cape St John Group is similar to calc-alkalic rocks associated with modern island arcs, although there is a predominance of high-silica rocks in the former; a marked compositional gap between 62 and 67% SiO_2 for the Cape St John succession corresponds to a lack of high-silica andesite and low-silica dacite and illustrates its bimodal nature. Sub- to low-greenschist facies metamorphism has affected the entire volcanic pile, and the metamorphic grade gradually increases from the southern part of the peninsula to the north, where it reaches amphibolite facies. An east-west trending syncline forms the major structure in the area and at least four phases of folding have been documented⁶. The Cape St John Group rests unconformably on the Snooks Arm Group (Arenig)^{7,8} and should, therefore, be younger than about 500 Myr.

We analysed 16 whole-rock samples of the Cape St John Group, for Rb, Sr and Sr isotopic composition (Fig. 2). All except two of these samples fall into two distinct groups. Group A defines an isochron corresponding to an age of 520 ± 40 Myr (mean square of weighted deviates, MSWD = 1.1)

whereas Group B gives an age of 385 ± 15 Myr (MSWD = 1.4). These ages are computed using the new decay constant for ^{87}Rb of $1.42 \times 10^{-11} \text{yr}^{-1}$ and the uncertainties are given at the 2σ level^{9,10}. Those samples with the higher $^{87}\text{Rb}/^{86}\text{Sr}$ ratios (> 2) lie along the line with lower slope, and yield an age which is significantly younger, by about 130 Myr, than the age recorded by the remainder of the samples. Those samples with low $^{87}\text{Rb}/^{86}\text{Sr}$ ratios retain an older age which we consider to be a very close approximation to the true age of the succession. Two of the data points shown in Fig. 2 have been excluded from the calculations because they fall well off the isochrons, as indicated by the large increase in MSWD observed when they are included. For Group A, the MSWD rises from 1.1 to 9.2, for Group B from 1.4 to 13.5.

We analysed all of our samples for all major elements to see if we could find any distinct groupings in addition to those that were based on the Rb/Sr ratios. We found: (1) Group A samples have SiO_2 contents between 44 and 69%, most have less than 2.5% K_2O (mean 1.8%), and their $\text{Fe}^{3+}/\text{Fe}^{3+} + \text{Fe}^{2+}$ ratios are normally less than 0.65; (2) Group B samples have greater than 70% SiO_2 , greater than 3.5% K_2O (mean 4.0%) and $\text{Fe}^{3+}/\text{Fe}^{3+} + \text{Fe}^{2+}$ ratios usually above 0.65. Two well-defined

fields on a plot of mole per cent $(\text{Na}^+ + \text{K}^+)$ against Mg^{2+} against $(\text{Fe}^{3+} + \text{Fe}^{2+})$ correspond to our initial groupings (see Fig. 2). These results clearly show that the two groupings are related to differences in bulk chemical composition, and that the samples which have been reset correspond to the more silicic members of the succession.

Evidence for open-system behaviour is not restricted solely to our Rb/Sr data. The alkali metals, DeGrace *et al.*⁶ believe, have been remobilised and redistributed during metamorphism "especially in the acid rocks". A negative correlation between Na and K in the more silicic rock contrasts with a positive correlation for the more basic and intermediate members of the succession.

The distribution of the data points in Fig. 2 can be explained in at least three different ways. Most of the Group B samples were collected in the northern part of the area and thus were subjected to a higher grade of metamorphism. Although we cannot entirely rule out change in metamorphic grade as one possible explanation for the distribution of the data, the fact that samples were collected from a relatively small area (32 km²) makes us think that it would be fortuitous for a critical isotherm to exist independently exactly at the junction between two quite

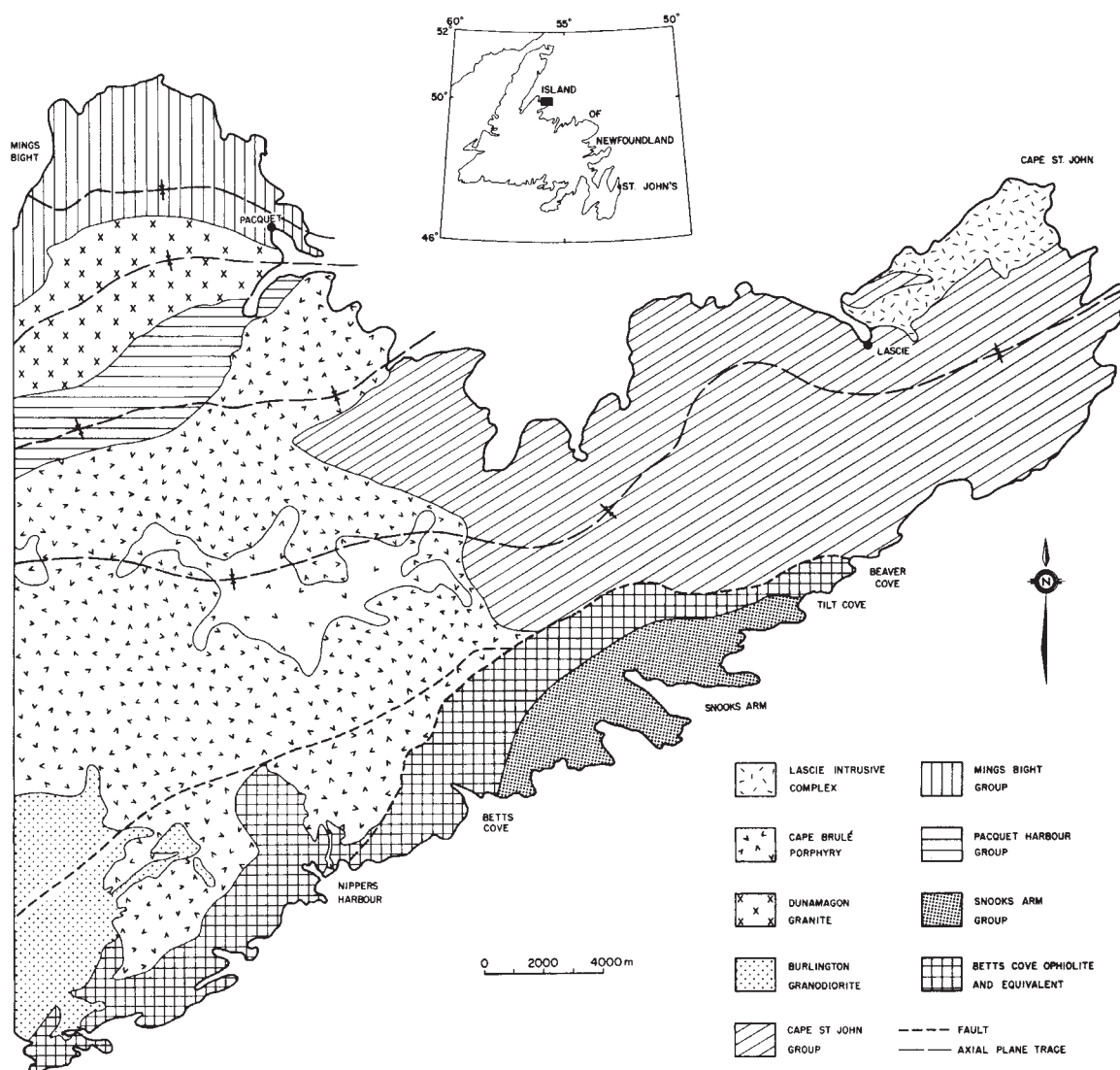


Fig. 1 Geology of the eastern part of the Burlington Peninsula, Newfoundland. Based on Map (2E/13), Geology of the Nippers Harbour Map Area, 1976, Newfoundland Dept of Mines and Energy.

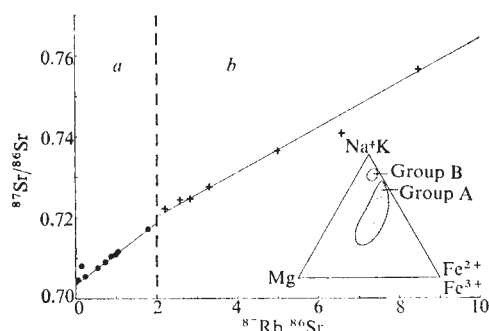


Fig. 2 Plot of $^{87}\text{Sr}/^{86}\text{Sr}$ against $^{87}\text{Rb}/^{86}\text{Sr}$ data for the Cape St John samples. *a*, Group A samples (●); *b*, Group B (+). Inset shows a plot of mole per cent Na+K against Mg against total Fe.

chemically distinct groups of rocks. One sample, in fact, that belongs to Group B was collected to the south of the area from which most of the Group A samples came. In addition, any evidence for variation in metamorphic grade from one group to another cannot be recognised in thin section examination. Another possible explanation, that the Group B rocks are representative of a much younger suite of volcanic rocks, is not supported by recent investigations of that area⁶. Many of the individual units of both basic and silicic composition are shown to interfinger, all seem to belong to the same stratigraphic succession, and no major unconformities can be found that separate the more silicic rocks from the others. DeGrace *et al.*⁶ emphasised that no major tectonic junctions were discovered within the Cape St John Group. The third interpretation, which we favour, involves a metamorphism some 390 Myr ago, that reset part of the Rb/Sr system. We suggest that only the more silicic parts of the succession were affected, and that their behaviour as open-chemical systems, at least with respect to Rb and Sr, was controlled by bulk chemical composition.

Support for our interpretation of the Rb/Sr data is given by results from two recent geochronological studies. $^{40}\text{Ar}/^{39}\text{Ar}$ work on rocks from northeastern Newfoundland has documented thermal disturbances in areas that lie to the east and the west of the Baie Verte lineament¹¹; the mineral ages to the east of the lineament range from 364 to 342 Myr; ages distinctly higher are found to the west (420–368 Myr). Although discordant, these ages mostly agree with the younger recent at 385 Myr documented by the Rb/Sr data from our Group B samples. Furthermore, slightly discordant U/Pb zircons¹², separated from a silicic pyroclastic, can be interpreted to indicate an age of about 550 Myr for the Cape St John volcanics, which is similar to our value of 520 ± 40 Myr.

Some important implications that relate to the dating of meta-volcanic rocks can be drawn from the Rb/Sr patterns shown in Fig. 2. If, for example, both the basic and silicic members of the succession had been used to define a single isochron, an 'age', albeit with a large uncertainty, would have been obtained which had little geological significance, though such a value would normally be intermediate between the age of the succession and the age of metamorphism. In addition, the closed-system behaviour for the more basic rocks of the Cape St John Group (at least for Rb and Sr) suggests that the true age of the succession may still be retrieved from metavolcanic material, while data from the more silicic members may be useful in determining the age of the last thermal event. However, full advantage can be taken of these differences if an evaluation is made of the whole-rock chemistry of the individual samples. Clearly, Rb/Sr ratios alone should not govern the final choice of samples for Rb/Sr isochron work.

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Simulated sunflecks have large, rapid effects on plant stem extension

CONTINUOUS simulated shade light quantity¹ and shade light quality² both modulate plant stem extension. However, in nature, shade light is not static, but is invariably, periodically interrupted by sunlight, in a manner primarily dependent on vegetation density and canopy structure^{3–5}. The significance of 'sunflecks' to growth and development of shaded vegetation remains almost unknown. Certainly in etiolated tissue, abnormal changes of light quality have been found to mediate very rapid changes of hypocotylar extension rate⁶, and in green tissue of deeply shaded plants the maintenance of photosynthesis above the compensation point may be highly dependent on light from sunflecks⁵. Furthermore, after very long treatment periods, developmental changes have been observed in response to short term light/dark alternations⁷. We report here that very brief changes of the light environment, simulating sunflecks, can elicit rapid, marked, changes in extension rate in mature green plants.

Sunflecks were simulated by instantaneous switching between low irradiance white + far-red light (LWFR), and high irradiance white light (HW) without added far-red (Fig. 1). This simulation is probably the most complete yet attempted, but it lacks some precision in the blue region of the spectrum; at the same time, however, the simulated environment accurately reflects the two other major parameters of sunfleck and shade—irradiance and ξ .

The 660 nm : 730 nm ratio of spectral photon flux (ξ) was used for the description and simulation of shade density for two reasons. First, differential attenuation of the red and far-red wavebands by shading vegetation is a major characteristic of shade light^{8,9}. Second, 660 nm and 730 nm are the action maxima of the plant photomorphogenic pigment, phytochrome, which is probably responsible for detection of shade light quality².

The transducer (ND2/2.5 mm; Sangamo Western Controls) was mounted on a Perspex gantry, of the type designed by Gallagher *et al.*¹⁰, such that the armature was counterbalanced by 3.25 g. The plant was connected to a brass chain extension of the armature by tailors' thread,

which was tied below the apical bud. In all light treatments the leaf temperature, which was sensed by a thermistor contacting the abaxial leaf surface, was kept constant by a system of thermostatted hot plates and asbestos shields. White light was supplied from up to nine clear 150-W tungsten filament lamps, whilst the far-red light was filtered from up to 25 similar lamps by a sandwich of one layer of 3.15 mm thick green Perspex, and one layer of 3.15 mm thick red Perspex. All lamps were water-cooled, the water also acting as an infrared (heat) filter. Light treatments could be changed instantaneously by means of a remote

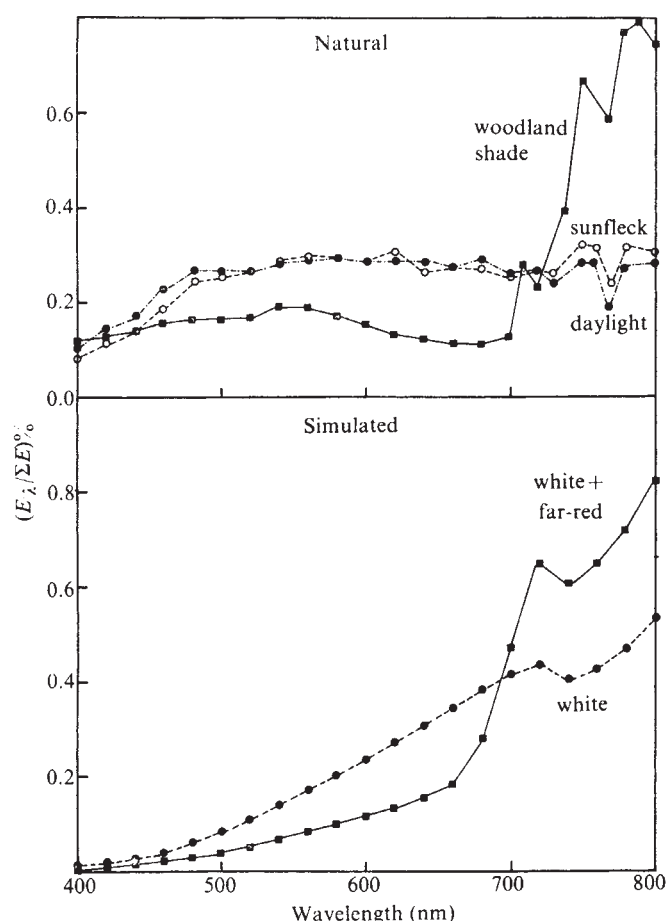


Fig. 1 Comparison of the simulated natural spectra with those from nature. The ordinate is expressed as the photon fluence rate at any wavelength (E_λ) as a percentage of the waveband total.

Light source	Date	Time (GMT)	PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	ζ
Simulated HW	—	—	119	0.790
HWFR	—	—	119	0.300
LW	—	—	25	0.791
LWFR	—	—	25	0.238

Natural

Natural Daylight	7.7.76	14:35	925	1.15
Sunfleck	8.7.76	13:00	121	1.04
(mature oak woodland)				
Woodland	7.7.76	12:50	24.4	0.28
(sub-mature/mixed)				

The woodland and daylight values of ζ are typical, but, of course, PAR can vary with the sky condition, in this case it was a cloudless day. PAR and ζ can vary depending on position within the sunfleck; these values, taken towards the periphery, are near maximal for this cloudless day. The relative levels of blue and red light in sunflecks can also vary considerably, but generally the blue fraction is smaller.

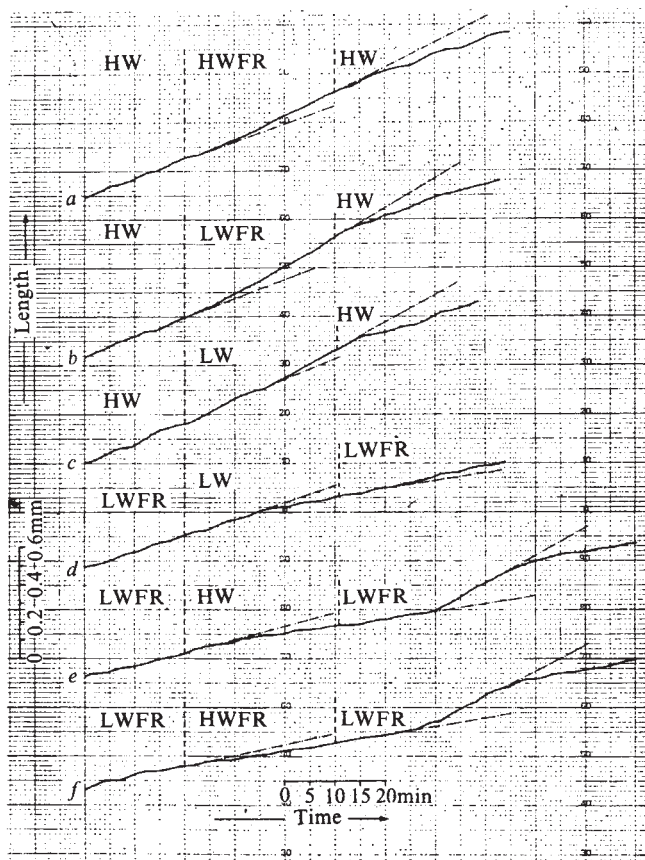


Fig. 2 Typical traces of the stem length/time relationship of *C. album* subjected to a 30-min change of light environment. Vertical dashed lines indicate the point at which the light environment was altered. Dash and dot continuation lines were drawn by eye.

switch box and dimmer switches. The complete system has been described in more detail by Morgan¹¹.

Plants of the arable weed *Chenopodium album* L. were kept for 1 week at a given level of photosynthetically active radiation¹² (either 25 or 119 $\mu\text{mol m}^{-2} \text{s}^{-1}$), after which a single plant (five nodes, 6.9 cm tall, and approximately 39 d old) was attached to the transducer and left to settle overnight (8 h dark/8 h light of either LWFR, if pre-equilibrated to 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$, or HW if pre-equilibrated to 119 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Extension was then monitored by the transducer, which within this system was accurate to within 3.4% of any measurement and gave a full scale deflection of the recorder for 2.62 mm displacement. The effect of a 30 min change of light regime was observed. Each possible combination of switches between equilibration HW or LWFR and treatment HW, LW, or LWFR was tested.

The results (Table 1) clearly demonstrate the rapidity, and high variability, with which *C. album* responds to a change in light environment. Most straightforward of the responses are those from plants equilibrated in HW, which simulates open conditions, followed by a brief transfer to simulated shade conditions (Fig. 2a-c). Here the lag period before response was between 7 and 11 min, both switching from, and returning to the HW environment. Either or both shade light characteristics (low irradiance, low ζ) induced an increase in the rate of extension, but the complete shade simulation (LWFR) elicited the maximum response. On return to the HW regime, only the extension rate of the plant which had been treated with LWFR was greater than the original HW extension rate; thus only these plants gain

Table 1 Effect of a 30-min change of ζ and/or PAR on the pattern of stem extension in *C. album* equilibrated to either LW or LWFR

Light environment Equilibration	Treatment	No. reps	% Change of Extension Rate		Lag period (mins)	
			Equil. $\rightarrow$ treatment	Treatment $\rightarrow$ equil.	Equil. $\rightarrow$ treatment	Treatment $\rightarrow$ equil.
HW	HWFR	12	+23.4 $\pm$ 3.4	-26.8 $\pm$ 3.5	7.4 $\pm$ 0.9	8.1 $\pm$ 1.3
	LWFR	12	+50.0 $\pm$ 12.0	-44.4 $\pm$ 3.16	11.7 $\pm$ 1.1	7.0 $\pm$ 0.8
	LW	11	+24.9 $\pm$ 5.3	-29.8 $\pm$ 3.3	11.5 $\pm$ 1.9	—*
LWFR	LW	6	-34.0 $\pm$ 7.5	+43.2 $\pm$ 18.8	10.0 $\pm$ 4.5	6.5 $\pm$ 2.5
	HW	5	-47.9 $\pm$ 6.4	+332.0 $\pm$ 42.5	4.8 $\pm$ 0.7	17.6 $\pm$ 1.2
	HWFR	5	-29.8 $\pm$ 7.9	+217.9 $\pm$ 73.0	4.7 $\pm$ 0.7	11.8 $\pm$ 3.2

The values in the body of the table are the means $\pm$ s.e.m.. No. reps, the number of replicate plants tested per treatment.
 % Change of extension rate (Treatment $\rightarrow$ equilibration) for the treatments in which a compensatory burst is seen (LWFR $\rightarrow$ HW; LWFR $\rightarrow$ HWFR), is the percentage increase of extension rate between that during the treatment light regime and that of the compensatory burst.
 *This could not be measured on most traces because of the low signal/noise ratio.

an overall height increase after a single treatment. These responses, which are characterised by a short lag period and a relatively small (20–50%) change in extension rate, may be referred to as 'rapid modulation' responses.

When the shade-to-sunfleck transition was simulated, the results were rather more complex. The switch LWFR $\rightarrow$ LW (Fig. 2d) yielded results essentially similar to those found in the HW equilibration transitions, but when the switch was from LWFR to high irradiance, simulated sunfleck (HW or HWFR), the response was quite different (Fig. 2e, f). The switch to high irradiance brought about the expected small decrease in extension rate (HWFR < HW) after a lag of about 5 min. There was, however, no equivalent small increase of rate about 5 min after return to LWFR as might have been expected from the previous results; instead, a large 'compensatory burst' of extension occurred after a 12–20 min delay, lasting for about 15 min, after which the rate settled to a level slightly less than the original LWFR rate.

The 'compensatory burst' response seems to be, at least in part, different from the 'rapid modulation' responses, being transient, much larger (200–300% increase), and having a longer lag period. Change of light quantity is obviously the major, possibly the sole, determinant of this response. On the other hand, the rapid modulation type responses seem to be equally sensitive to change in light quality and quantity. Light quality changes were produced by changing the R/FR balance, which may mean that phytochrome acts as the responsible photoreceptor.

These data demonstrate that the transient changes which occur in the natural light environment can significantly modulate plant development. More account should therefore be taken of sunflecks when attempting to relate the growth and development of plants to environmental factors.

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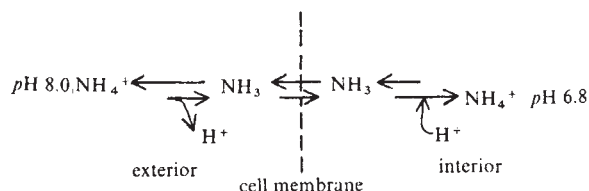
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Mechanism of action of NH_4Cl and other weak bases in the activation of sea urchin eggs

EXPOSURE of unfertilised sea urchin eggs to NH_4Cl and other weak bases such as procaine or nicotine results in the activation of some of the events which normally follow fertilisation, such as the acceleration of protein synthesis and the initiation of DNA synthesis^{1,2}. The initial events of normal fertilisation, for example, the cortical reaction and respiratory burst, are bypassed. It has been shown that fertilisation triggers a Na–H ion exchange that results in an increase in the intracellular pH and a decrease in the pH of the surrounding seawater³. There is a similar acidification of the seawater and increase in intracellular pH when sea urchin eggs are exposed to NH_4Cl or procaine. This led to the suggestion that the pH changes induced by weak bases were mediated by a mechanism similar to the Na–H ion exchange. However, it had been proposed that NH_4Cl worked by a simple passive diffusion mechanism, where the uncharged species of weak base diffuses into the egg and picks up an H^+ ion, resulting in an increase in the intracellular pH (ref. 4). The important distinction between these two mechanisms is that if weak bases are working by a passive diffusion mechanism, bypassing the Na–H ion exchange, then their action gives direct support to the hypothesis that an increase in the intracellular pH plays a part in the onset of biosynthetic activities after fertilisation. We show here that weak bases bypass the Na–H ion exchange, cause both the internal and external pH changes directly by their passive diffusion into the egg, and that the initial increase in the rate of protein synthesis is proportional to the amount of weak base entering the egg.

The following scheme adapted from Jacobs demonstrates how weak bases directly cause both the internal and external pH changes⁵.



The charged and uncharged species of weak base are in equilibrium in seawater. When sea urchin eggs are added, the uncharged species diffuses into the egg until the concentration of uncharged weak base inside is equal to that outside.

The egg is only permeable to the uncharged species of weak base⁵. To maintain the equilibrium, the charged species outside give up protons, resulting in a pH decrease. The uncharged form, once in the cytoplasm, accepts protons, resulting in an increase in the intracellular pH. As the intracellular pH is at least two pH units below the pK_a of NH_4Cl (9.25) or procaine (8.95), more than 99% of the uncharged base that enters the aqueous compartment of the egg will pick up a proton (ref. 6; we assume that the ideal value for the pK_a 's of NH_4Cl and procaine are not greatly altered in seawater). Thus, the generation of acid when eggs are added to seawater containing a penetrating weak base is not caused by protons released from the egg, but is due to maintenance of the equilibrium between the charged and uncharged species in the external medium.

There are several observations that support this passive diffusion mechanism. NH_4Cl and other weak bases cause both the intracellular pH change and the activation of the egg in Na-free seawater^{3,7}. The NH_4^+ ion is not substituting for Na^+ in the Na-H ion exchange because the amount of H^+ generated is dependent on the uncharged rather than the charged concentration of weak base as described below. When eggs that have come to equilibrium with weak base are transferred to fresh seawater, the external pH increases. This would be expected if the uncharged form passively diffused out of the egg and picked up protons as equilibrium is approached.

Quantitative measurements show that there is close to a 1 : 1 ratio between the entry of weak base into the egg and the H^+ generated with procaine. Figure 1 shows the relationship between the accumulation of ^{14}C -procaine and the concurrent change in external H^+ concentration at pH 8.0. The small

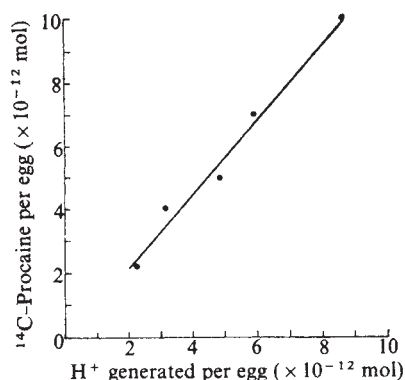


Fig. 1 Relationship between the entry of ^{14}C -procaine and the change in external H^+ concentration. To determine the H^+ generation, 50 μ l of packed, dejellied *Lytechinus pictus* eggs were added to 10 ml of seawater containing varying concentrations (0.25–5 mM) procaine at pH 8.0. The change in external pH was measured and the number of H^+ generated was determined by back titration with 0.001 N NaOH of a duplicate sample without eggs over the same pH range. The accumulation of ^{14}C -procaine (New England Nuclear, 46.8 mCi mmol⁻¹) was determined by adding 50 μ l of packed, dejellied eggs from the same female used to measure the generation of H^+ ions to 10 ml of seawater containing various dilutions (0.25–5 mM) of a stock solution of 10 mM procaine and 0.1 μ Ci ml⁻¹ ^{14}C -procaine. At 5, 10 and 15 min, a 2-ml aliquot was removed and washed once with ice-cold seawater containing an equal concentration of unlabelled procaine. The eggs were then dissolved in NCS and the amount of radioactivity determined by liquid scintillation counting. The predicted ratio of generation of H^+ ion in the external medium to the entry of weak base was calculated as follows. From the Henderson-Hasselbalch equation, where u is the amount of uncharged weak base, c the amount of charged weak base, and x the amount of weak base that loses a proton to replace one molecule of uncharged weak base that enters the egg, or the amount of H^+ generated, then, $pH = pK_a + \log (u - 1 + x) / (c - x)$ and, $x = (10^{pH - pK_a} c - u + 1) / (10^{pH - pK_a} + 1)$. Assuming the change in external pH is small, then $10^{pH - pK_a} c - u = 0$, and $x = 1 / 10^{pH - pK_a} + 1 =$ amount of H^+ generated per entry of one weak base molecule.

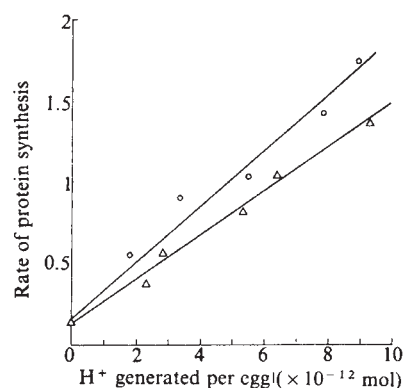


Fig. 2 The relative rate of protein synthesis as a function of varying concentrations of NH_4Cl or procaine. $\circ$, NH_4Cl ; $\triangle$, procaine. 0.75 ml of packed, dejellied eggs were preloaded with 5 μ Ci ml⁻¹ of 3H -valine (Schwarz-Mann, 26 mCi mmol⁻¹) in seawater for 45 min, washed 3 times in ice-cold seawater, and then 50 μ l of packed eggs were added to 50 ml of seawater containing various concentrations of NH_4Cl or procaine at pH 8.0. After 15 min, the eggs were washed twice with fresh seawater, and resuspended to the original concentration. 2-ml aliquots were taken every 10 min, dissolved in sodium dodecyl sulphate (SDS) (final concentration, 0.5% SDS containing 0.1 mg ml⁻¹ bovine serum albumin) and precipitated with an equal volume of ice-cold 20% trichloroacetic acid (TCA) containing 2 mg ml⁻¹ unlabelled valine. The precipitates were collected on glass fibre filters, washed 3 times with 5% TCA containing 1 mg ml⁻¹ unlabelled valine, once with water and twice with methanol. The filters were air dried, 5 ml of NCS-omnifluor scintillation fluid was added, and the samples were counted. The relative rate of protein synthesis was calculated as Δ c.p.m./time. The change in the external H^+ concentration for varying concentrations of procaine and NH_4Cl was determined as in Fig. 1.

deviation from the 1 : 1 ratio between procaine accumulated and H^+ generated in the external medium is due to the fact that at pH 8.0 a substantial proportion (10.1%) of the procaine molecules in the external medium exist as the uncharged species. Consequently, not all the procaine molecules which enter the egg will generate protons in the external medium. The predicted ratio of H^+ generated in the external medium to procaine molecules which have entered the egg is 0.90 : 1. Figure 1 shows that the experimentally determined value is 0.85 : 1. This is within twice the standard deviation. The fact that the H^+ generated depends on the ratio of charged to uncharged weak base as well as the concentration of uncharged weak base is the strongest demonstration of the validity of the passive diffusion mechanism. Thus, the amount of weak base entering the egg can be calculated from measurements of the H^+ generated in the external medium. (For details of calculation, see legend of Fig. 1.)

We determined the relationship between the stimulation of the initial rate of protein synthesis and the amount of weak base entering the egg. Unfertilised eggs have a very low rate of protein synthesis. Shortly after fertilisation or exposure to weak base, there is an increase in the rate of protein synthesis¹. Figure 2 shows that the rate of protein synthesis caused by a brief exposure to NH_4Cl or procaine is directly proportional to the magnitude of the change in the H^+ concentration outside the egg, and thus to the amount of uncharged weak base entering the egg. The small differences observed in the efficiency of NH_4Cl and procaine in stimulating protein synthesis may be due to the fact that, in theory, a given uncharged concentration of NH_3 will lead to a greater increase in intracellular pH than will procaine.

The simplest interpretation of this data is that weak bases activated protein synthesis directly by raising the intracellular

pH. However, the kinetics of the actual intracellular pH change and the change in the rates of protein synthesis make it difficult to show direct quantitative links between the two. We find that NH_4Cl and procaine equilibrate very quickly with the inside of the sea urchin egg, that is, with 5 mM procaine, the generation of H^+ ions in the external medium is complete within 3 min. As almost every weak base molecule that enters the egg picks up a proton, an immediate rise in the intracellular pH would be expected. However, the intracellular pH change measured directly has a much longer time course, that caused by 5 mM procaine requiring 15 min for completion⁸. This indicates that the intracellular pH is regulated by the egg in response to passive changes in the internal H^+ concentration imposed on the cell by the influx of weak bases. At this time, we cannot quantitatively relate changes in intracellular pH to the rate of protein synthesis, because of the complex response of intracellular pH to the weak base stimulus. However, it is clear that the effect of the penetration of the uncharged form of the weak base is to raise the intracellular pH, which results in an increase in the rate of protein synthesis.

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Effect of cocultivation on sister chromatid exchange frequencies in Bloom's syndrome and normal fibroblast cells

BLOOM's syndrome (BS) is a rare, autosomal recessive human disorder, characterised by growth retardation, sunlight-induced facial skin eruptions, low immunological competence and a high incidence of cancer^{1–4}. Lymphocytes, and to a lesser extent, fibroblasts, cultured from affected individuals exhibit unusually high levels of chromosomal aberrations^{2,5,6} and sister chromatid exchanges (SCE)^{7,8}, suggesting that BS cells may be defective in DNA repair and/or synthesis^{9–11}. However, BS cells are normally competent in excision repair^{11–13}, postreplication repair¹⁴, single-strand break rejoining¹⁴, and have normal levels of DNA polymerases (α , β , γ)¹⁵. On the other hand, these cytogenetic manifestations of BS could indicate the presence of actual DNA damage, perhaps a consequence of some altered metabolite(s) produced endogenously. To examine this possibility, we assessed the effect of cocultivation of fibroblast cells from a BS individual with those from an unaffected control on SCE frequencies in both cell populations. Results of these studies indicate that BS cells produce an agent(s) capable of causing an increased SCE response in normal cells.

Table 1 Growth characteristics of fibroblast cell lines

Cell lines	PE (%) [*]	PDT (h) [†]	SD (10^4 cm^{-2}) [‡]	Cell kinetic distribution§		
				X1	X2	X3
BS (1492)	50.1 ± 4.9	28.5 ± 2.9	3.73 ± 0.57	8	92	0
CM	50.3 ± 2.1	25.3 ± 2.1	3.50 ± 0.20	5	95	0
CF	51.2 ± 8.2	26.0 ± 2.6	3.81 ± 0.12	4	95	1

Fibroblast cell lines were cultivated in Eagles minimal essential medium (MEM) with Earle's salts (Gibco), 20% fetal calf serum (Gibco), 2 mM L-glutamine, penicillin ($10,000 \text{ IU ml}^{-1}$), streptomycin ($10,000 \mu\text{g ml}^{-1}$) (Gibco) at 37 °C and 7.5% CO_2 . The BS cell line (GM1492) was male in origin and was obtained from the Human Genetic Mutant Repository, Camden, New Jersey, at passage level 12 and used at passage levels 16–25. Both control skin fibroblast cell lines were of embryonic origin and were used at passage levels 10–20. Plating efficiency, population doubling time and saturation density were calculated from growth curves on semi-logarithmic plots obtained from three separate experiments. In each case, $2-3 \times 10^3 \text{ cells cm}^{-2}$ were inoculated into 25- cm^2 tissue culture flasks (Falcon), the number attached cells counted at 18 h, and daily cell counts in triplicate taken for the next 6 d. Cultures were fed at 18 h after initiation and subsequently every other day. Cells were collected by combined treatment with trypsin-EDTA (Gibco) and a rubber policeman. Cell counts were determined on a Coulter Counter, Model ZBI (Coulter). To compare cell proliferation kinetics, duplicate samples of about $10^4 \text{ cells cm}^{-2}$ were inoculated into 75- cm^2 tissue culture flasks (Falcon) in medium containing 25 mM BrdU (Sigma). Average values were derived from four separate experiments. Colcemid (Gibco) was added for 2 h after 66 h of culture and the cells were collected and slides prepared as described previously¹⁹. The proportion of first (X1), second (X2) and third (X3) generation metaphase cells was determined for 100 consecutive metaphase cells in each culture as described previously¹⁹. Values are mean $\pm$ s.e.m.

^{*} Plating efficiency (PE) as percentage attached cells.

[†] Population doubling time (PDT) as calculated during logarithmic growth.

[‡] Saturation density (SD), maximum number of cells attained per unit area.

[§] Mean proportion of first (X1), second (X2), and third (X3) generation metaphase cells in 68 h cultures grown in the presence of 25 μM BrdU.

Table 2 Cocultivation experiments

Experi- ment	Cell type [*]	Not cocultivated SCE frequency	Cocultivated SCE frequency	Per- centage change	Prob- ability [†]
Control-Control					
1	CF	5.9 ± 0.2 (100)	5.3 ± 0.2 (100)	−10.2	0.084
	CM	6.8 ± 0.3 (100)	6.9 ± 0.2 (100)	+ 1.5	0.941
BS-Control					
1	CF	8.5 ± 0.3 (100)	11.7 ± 0.5 (65)	+ 37.6	0.000 [‡]
	BS	83.9 ± 2.7 (49)	79.7 ± 2.1 (62)	− 5.0	0.220
2	CF	5.7 ± 0.5 (21)	8.7 ± 0.3 (132)	+ 52.6	0.000 [‡]
	BS	56.3 ± 1.7 (44)	59.8 ± 1.6 (96)	+ 6.2	0.152
3	CF	5.9 ± 0.2 (100)	7.5 ± 0.2 (357)	+ 27.1	0.000 [‡]
	BS	79.9 ± 2.6 (37)	71.7 ± 2.3 (71)	−10.3	0.025
4	CF	6.5 ± 0.3 (127)	9.1 ± 0.3 (119)	+ 40.0	0.000 [‡]
	BS	59.4 ± 1.3 (102)	57.9 ± 1.4 (94)	− 2.5	0.446
Average alteration control cells				+ 39.3	
BS cells				− 2.9	

Cultures were grown as described in Table 1 for cell proliferation kinetic determinations, except that for cocultivation about $5 \times 10^3 \text{ cells cm}^{-2}$ of each cell type were inoculated into 75- cm^2 tissue culture flasks. After termination and slide preparation, second generation metaphase cells were examined for origin and SCE numbers determined for those cells unequivocally identified. BS and control cells could be differentiated not only on the basis of male against female karyotype, but also due to the presence of chromosomal polymorphisms in the D and G chromosome groups. SCE determinations were verified by two independent observers. The cell kinetic index was determined from 100 metaphase cells for which cell line of origin could be determined. See text for cell type designations. Values are means $\pm$ s.e.m.; numbers of analyses shown in parentheses.

^{*} Ratio of BS to control (CF) or control to control cells was at 1:1 at inoculation.

[†] Probability value derived from two-tailed probability *t* test using separate variance estimate.

[‡] Significance at $P \leq 0.01$.

Table 3 Effect of cocultivation on aberration frequency in BS and control fibroblast cells

Cell type	Cocultivation*	Cell number	Achromatid lesions	Chromosome aberrations				PCC†	% Damaged cells
				Chromatid breaks	Iso-chromatid breaks	Dicentric chromosomes	Tri-radials		
CF	—	250	26	8	0	0	0	0	12.8
	+	250	27	5	0	0	0	0	12.8
BS	—	250	42	27	24	3	2	10	28.8
	+	250	49	20	29	2	0	15	31.6

Cultures were grown as described in Tables 1 and 2, but in the absence of BrdU. Metaphase cells were cytogenetically identified as to origin and examined for chromosomal aberrations according to the criteria of Bender *et al.*²⁰. PCC were defined according to the criteria of Sperling *et al.*⁸. However, in each cell the PCC completed the normal complement and did not seem to represent a fragment but rather an entire chromosome. Comparison of data by a two-tailed probability *t* test assuming significance at the $P \leq 0.05$ level resulted in no significant differences.

* Ratio of control to BS cells was 1:1 at inoculation and 1.1:1 at termination.

† Premature chromosome condensation.

The validity of cocultivation experiments depends to a large extent on comparable growth kinetics for the cell lines used. Various BS and normal fibroblast cells were examined for their growth characteristics—plating efficiency, population doubling time, and saturation density. Based on similar growth characteristics (Table 1), one affected cell line (BS), one male control cell line (CM) and one female control cell line (CF) were chosen for cocultivation. The kinetic distribution of first (X1), second (X2) and third (X3) generation metaphase cells was then determined for each of these cell lines to ensure equal cell cycle kinetics in the presence of bromodeoxyuridine (BrdU) (Table 1).

SCE frequencies were determined for BS and CF cells cultivated separately and together. Cocultivation of equal numbers of BS and CF cells significantly increased the number of SCE in the CF cells, but had no effect on SCE levels in BS cells (Table 2). Comparable growth characteristics were verified internally for the cocultivated populations by cytogenetic determination of the ratio of male (BS) to female (CF) cells at termination. Cocultivation had no effect on cellular kinetics or on the ratio of BS to CF cells (data not shown).

To determine whether the observed increase in SCE frequency in the cocultivated CF cells was BS cell-dependent, two control cell lines (CM and CF) were cocultivated and SCE frequencies assessed. The results of this experiment demonstrated that cocultivation of control cells had no significant effect on SCE levels (Table 2).

BS and CF cells were then cocultivated in the absence of BrdU and the frequency of chromosomal aberrations compared. Cocultivation did not significantly alter the number of chromosome aberrations in either cell population (Table 3). This is not surprising, as SCE seems to be a much more sensitive indicator of certain types of DNA damage than chromosomal aberrations^{16,17}.

The BS cell-dependent increase in SCE frequency in control cells could be due to physical cell-cell contact or medium mediated. These alternatives were tested with PHA-stimulated human lymphocytes which were exposed for approximately two cell cycles to media collected from control and BS fibroblast cultures (Table 4). The demonstration of a significant increase in SCE in lymphocytes cultured in BS-derived medium above control cell-derived medium, suggests that the observed alteration in SCE frequency in cocultivated CF cells is due to a diffusible agent(s) present in medium.

Our results suggest that the defect inherent to BS cells involves the production of an agent(s) capable of damaging DNA. The increased level of both SCE and chromosomal aberrations are then the consequence of this damage, suggesting that BS cells may represent a normally stressed cell population. Several

observations are consistent with this interpretation. Although BS cells are normally competent in ultraviolet light UV-induced excision and postreplication repair^{11–14}, these cells have been reported to show diminished cell survival in response to this agent¹¹. Furthermore, although no deficiency in DNA polymerase activity has been observed¹⁵, DNA chain growth rate and maturation is depressed compared with control DNA^{10,11}. The distribution and types of chromosome aberrations present in BS cells are strikingly similar to those produced by the bifunctional crosslinking agent, mitomycin C (refs 3 and 18), and suggests that the DNA damaging agent(s) produced by BS cells may be a similarly reactive compound(s). We are currently investigating the nature of this active substance and are examining whether other genetic syndromes exhibiting high incidences of cancer may have similarly stressed cell populations.

Table 4 Effect of culture medium on SCE frequency in PHA-stimulated human lymphocytes

Experiment	Medium origin	SCE frequency $\bar{X} \pm S_x$ (n)	Percentage change	Probability*
1	CF	11.0 $\pm$ 0.7 (50)		
	BS	19.6 $\pm$ 2.2 (50)	+78.2	0.000†
2	—	6.0 $\pm$ 0.3 (60)		
	CF	6.3 $\pm$ 0.2 (150)		0.370
	BS	8.1 $\pm$ 0.3 (150)	+28.6	0.000†

Heparinised peripheral blood was obtained from one donor and whole blood cultures were incubated with PHA-M (Gibco) as described previously¹⁹, except for the presence of 25 μ M BrdU. Forty-eight hours after initiation, cultures were centrifuged and one-half of the medium replaced with BS fibroblast derived medium, control fibroblast derived medium or the original lymphocyte culture medium. All manipulations took place in low-light conditions. Cell cultures were incubated for an additional 24 h, approximately two cell cycles in these conditions¹⁹. Colcemid was present for the last 2 h. Cultures were processed, slides prepared and metaphase cells analysed for SCE as described previously¹⁹. Media from BS and control fibroblast cultures were removed from cells cultured as described in Table 2 but in the absence of BrdU, centrifuged to remove cells and cell debris, refrigerated and used within 24 h. Experiment 2 was modified in that the fetal calf serum for cell culturing had been heat-inactivated at 56 °C for 30 min. Data for experiment 1 was obtained from duplicate cultures (25 cells each) and for experiment 2 from duplicate cultures for the control (30 cells each) and from quintuplet cultures for the BS and control fibroblast medium cultures (30 cells each). SCE frequency given as mean $\pm$ s.e.m.; no. of analyses given in parentheses.

* Probability value derived from two-tailed probability *t* test using separate variance estimate.

† Significance at $P \leq 0.01$.

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Are T-cell lymphomas immunocompetent?

TUMOURS of the lymphoid system are generally assumed to be 'frozen' in permanent states of differentiation and cellular activation. However, we show here that thymic lymphoma cells of at least one cell line maintain their immunocompetence and can be specifically activated on contact with antigen in a manner analogous to that of normal, non-transformed T cells. Although T cells are known to express antigen binding receptors consisting in part of antibody variable regions^{1,2} which may be translated from mRNA in the cytoplasm³, the mechanisms controlling the induction of these V-region genes remain unknown. Furthermore, the events controlling the antigen-dependent activation of T cells expressing specific receptors are poorly understood, due largely to the unavailability of homogeneous monoclonal T cells. It is likely that the production of antigen-specific T-cell lines will be useful for investigations into the molecular biology of T-cell activation and differentiation, just as the myelomas have been indispensable in elucidating B-cell function and control.

We used a leukaemia virus originally isolated from a thymic lymphoma induced by the neonatal injection of 7,12-dimethylbenzanthracene into CFW/D mice⁴. The virus was then injected into newborn thymus 7 d after grafting under the kidney capsule of adult mice, and the cell line (485-2) was established from a resulting lymphoma. After 53 passages, this line had 41 stable chromosomes, a doubling time of 14.5 h, and induced 100% 'takes' *in vivo* when as few as 5×10^3 cells were injected⁵. Supernatant fluids from the cell line contained infectious C-type virus particles which had a

buoyant density of 1.6 g cm^{-3} and contained reverse transcriptase activity as well as 60-70S viral RNA (J.K.B., in preparation). In addition, almost 100% of these cells were killed by treatment with specific anti-thymocyte serum and complement.

Cell transfer experiments revealed that virus-induced thymomas contained functional helper T cells. Adult mice were grafted under the kidney capsule with neonatal thymus and the graft was injected with saline as control or with virus from 485-2 culture supernatants. When the virus-injected graft became grossly enlarged, graft cells were transferred with sheep red blood cells (SRBC) and normal, syngeneic bone marrow cells into lethally irradiated recipients. As shown in Fig. 1, control cells or cells from virus-injected grafts cooperated with bone marrow cells to mount an anti-SRBC response which was severalfold higher than when either bone marrow or graft cells were transferred alone. This suggested that transformed T cells from the thymoma might possess normal immunocompetent function. However, additional experiments revealed that only 90% of the cells in the tumour were killed by treatment with

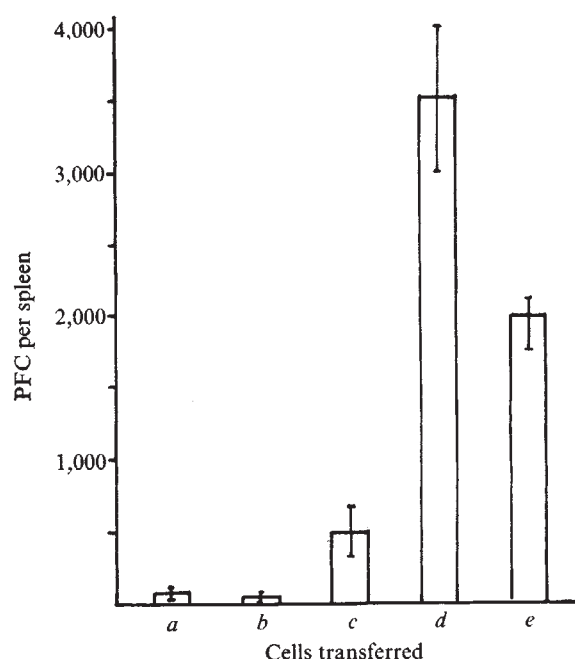


Fig. 1 Presence of functional helper cells in tumorous thymic grafts. 20×10^6 bone marrow cells and/or 10^8 thymus graft cells from saline or virus injected grafts, 60 d post-injection, were transferred with 4×10^8 SRBC into groups of lethally irradiated (850 R) syngeneic recipients. Values represent the mean PFC per spleen $\pm$ s.e.m. of four individual mice assayed 7 d after transfer. a, Virus injected graft; b, saline injected graft; c, normal bone marrow; d, normal bone marrow + virus injected graft ($P < 0.001$); e, bone marrow + saline injected graft ($P < 0.02$).

anti-thymocyte serum and complement. It could, therefore, not be ruled out that normal, infiltrating host cells were responsible for the cooperation with bone marrow cells noted in Fig. 1. To avoid this problem, we examined the ability of the 485-2 cell line to be directly activated by antigen.

It has been shown that T cells pre-exposed to the appropriate antigen in irradiated recipients are capable of cooperating with B cells in T-cell deprived cultures, thereby restoring the antibody response^{6,7}. As shown in Table 1, 485-2 cells activated with SRBC restored the anti-SRBC plaque forming cell (PFC) response to almost the same level as that of normal, activated thymocytes. This restoration was specific, as the use of horse erythrocytes (HRBC), or no antigen, during the activation step did not result in a PFC

Table 1 The activation of tumour cells with sheep erythrocytes

Additions to T cell deprived spleen cell cultures*	Plaque forming cells per 10 ⁶			
	anti-SRBC	P value†	anti-HRBC	P value
None	600 ± 52§	—	516 ± 86	—
485-2 cells activated with SRBC‡	1,800 ± 130	0.01	549 ± 43	NS
485-2 cells activated with HRBC	715 ± 68	NS	ND	—
485-2 cells (nonactivated)	540 ± 75	NS	ND	—
Normal thymocytes activated with SRBC	2,530 ± 210	0.001	690 ± 87	NS
Normal thymocytes activated with HRBC	720 ± 91	NS	2,021 ± 110	0.001

* Normal CFW/D spleen cells were treated with anti-thymocyte serum and complement before Mishell-Dutton culture at a concentration of 10⁷ per dish with 2 × 10⁷ erythrocytes. Untreated control cultures developed 3,010 PFC per 10⁶ against SRBC and 1,935 PFC per 10⁶ against HRBC.

† P values were determined by a Student's *t*-test. NS, not significant (*P* ≥ 0.05).

‡ 4 × 10⁷ normal thymocytes or 485-2 cells were injected alone (nonactivated) or with 4 × 10⁸ SRBC or HRBC into lethally irradiated (850 R), syngeneic recipients, and the spleens were used as a source of activated cells 6 d later. A predetermined optimum dose (10%) of these cells was added for culture.

§ Values represent the mean PFC per 10⁶ ± s.e.m. of triplicate cultures assayed individually on day 5.

|| ND, not determined.

response significantly above background levels. Furthermore, 485-2 cells activated with SRBC did not restore the anti-HRBC response.

In summary, we have shown that permanently transformed T cells of a leukaemia line (485-2) maintain their immunocompetence and can be specifically activated by antigen. Additional observations support the hypothesis that transformed cells can be induced to differentiate. For example, appropriate triggering signals may induce H-chain gene switching in B lymphoblastoid cell lines⁸ or haemoglobin production in erythroleukaemia cells⁹.

As T-cell activation by an antigen may involve differentiation events, these observations have important implications regarding current theories of oncogenesis. It is crucial, therefore, to examine the ability of additional cell lines to be activated by a wide variety of antigens, for one important question remains: did the 485-2 cell line grow out from those few transformed cells which were by chance specific for SRBC or do endogenous viruses enhance the generation of receptor diversity in lymphoid cells?

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Cytotoxic T lymphocyte response to murine cytomegalovirus infection

THE immune response to cytomegalovirus (CMV) infection is poorly understood, but it is believed that the cellular immune system has a critical role. It is a major cause of morbidity and mortality in homograft recipients^{1,2}, leukaemic individuals^{3,4} and others receiving immunosuppressive drug therapy⁵. In these patients, high levels of serum antibody often fail to eradicate chronic infection⁶. Infection of mice with murine cytomegalovirus (MCMV)⁷ has provided additional evidence of the importance of T-lymphocyte function in the immune response to CMV: nude mice⁸, and mice treated with anti-thymocyte serum⁹ or

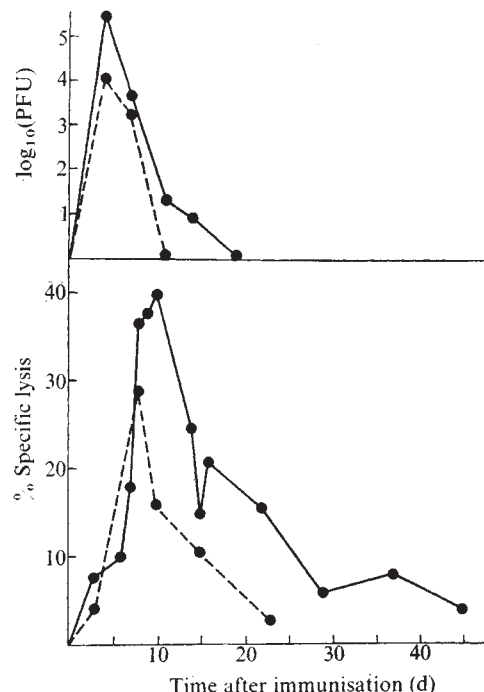


Fig. 1 Temporal characteristics of cytotoxic T-lymphocyte response shown in relation to infectivity of spleen cell suspensions. Weanling BALB/c mice were infected intraperitoneally (i.p.) with 1,000 (dashed line) or 10,000 (solid line) plaque forming units (PFU) of the Smith strain of MCMV¹³. Lymphocyte suspensions were prepared in Eagle's minimum essential media (MEM) supplemented to 10% with heat-inactivated fetal bovine serum (FBS) from spleens removed at various times after infection, then treated with ammonium chloride buffer to lyse erythrocytes¹⁴ and resuspended in media. For target cells, BALB/c MEC were infected with one PFU per cell of MCMV for 24 h, collected by trypsinisation, incubated for 30 min in media containing 100 μ Ci ml⁻¹ of ⁵¹Cr-sodium chromate (New England Nuclear), washed twice, and then resuspended in MEM with 10% FBS. 0.2-ml aliquots containing 10⁶ spleen cells and 10⁴ target cells were placed in round-bottomed microtitre plates (Linbro) and incubated for 20 h at 37 °C in a 5% CO₂ atmosphere. Supernatant was collected with a Titertek supernatant collection system (Flow) and released chromium measured with a γ counter (Searle model 1185). Spontaneous target cell lysis varied over 25–40% of maximum lysis. % Specific lysis values were calculated from the equation:

$$\% \text{ Specific lysis} = \left(\frac{\text{c.p.m. test} - \text{c.p.m. spontaneous}}{\text{c.p.m. maximum} - \text{c.p.m. spontaneous}} \right) \times 100$$

% Specific lysis values greater than 10% were always highly significant, but lower values often were not. All spleen cell suspensions were prepared from two spleens, and all tests were carried out in replicas of eight. Infectivity assays were carried out by plaque titrations of aliquots of spleen cell suspensions on monolayer cultures of BALB/c MEC using an overlay of MEM with 5% FBS and 0.5% Agarose.

undergoing graft-versus-host reaction¹⁰ all succumb to much lower infecting doses of virus than do normal mice. It has been shown that purified human CMV can induce proliferation of lymphocytes from immune individuals¹¹, but T-cell specific responses to CMV infection have not yet been demonstrated. Cytotoxic T-lymphocyte responses have been described for several viral infections¹²⁻¹⁷. We report here a virus-specific cytotoxic T-lymphocyte (CTL) response in MCMV infection.

The results of assays of spleen cell suspensions for infectious virus and CTL are presented in Fig. 1. Cytotoxic lymphocytes could be detected as early as three days after virus inoculation. Both infectious virus and CTL were present in greater amounts and persisted longer after the higher infecting dose of virus. Late values of 3-8% specific lysis were not significantly different from controls, and values greater than 10% were always highly significant. In experiments carried out with four separate groups of mice, cytotoxic spleen cells were detected from three days to a week after virus could no longer be cultured from spleen cell suspensions. These results indicate that the persistence of cytotoxic lymphocytes in spleens is related to, but not dependent on, the presence of infectious virus.

Several experiments were carried out to examine the properties of the effector spleen cells. Incubation of spleen

Table 1 Effect of pretreatment on lymphocytotoxicity

Pretreatment of effector cells	% Lysis
None	11%*
Nylon wool incubation	33%*
Anti- θ serum + complement	0%
Media + complement	16%*
Media + media	31%*

Spleens taken 15 d after i.p. infection with 1,000 PFU of MCMV. To deplete T cells, spleen cells were suspended in a 1:10 dilution of AKR anti- θ serum (prepared against C3H θ antigen) for 30 min followed by a 30-min incubation in a 1:4 dilution of rabbit complement. By Trypan blue exclusion it was shown that this treatment lysed approximately 30% of splenic lymphocytes. Suspensions in MEM with 10% FBS and 5×10^{-6} M 2-mercaptoethanol were enriched for T cells by incubation in a nylon wool column for 45 min at 37 °C in 5% CO₂ atmosphere; non-adherent cells were eluted by gravity drainage¹⁵.

*Significant differences between test and spontaneous lysis, $P < 0.01$.

cells in nylon wool to remove B cells and macrophages¹² yielded a suspension that was three times more cytotoxic than untreated immune spleen cells (Table 1). Conversely, T-cell depleted lymphocyte populations, obtained by treatment with anti- θ serum and complement, did not lyse target cells, but suspensions treated with complement alone or with media retained cytotoxic activity. As noted, spleen cells treated only with media were actually more cytotoxic than the original suspension. This observation was a reproducible effect of the two additional incubations at 37 °C with repeated washings, and may have resulted from removal of blocking factors¹⁶. The results of experiments demonstrating virus specificity of the effector cells are presented in Table 2, and evidence that the effector cells recognise only target cells sharing the same H-2 haplotype is presented in Table 3.

From these experiments we conclude that generalised infection of BALB/c mice with MCMV induces prompt differentiation of splenic lymphocytes into cytotoxic cells. This response is maximal 10 d after immunisation and progressively falls after elimination of infectious virus from the spleens. Firm evidence that these effector cells are T lymphocytes was provided by the demonstration that they

Table 2 Specificity of target cell lysis

Effector cell immunisation	Target cell infection	% Specific lysis
MCMV	MCMV	18.6-32.6%
MCMV	None	0.0%
MCMV	HSV-1	0.5%
MCMV	Influenza	0.0%
Influenza	MCMV	0.0%
Influenza	Influenza	11.7%

Spleens were removed from BALB/c mice 5-10 d after i.p. infection with 10,000 PFU of MCMV or 5 d after intranasal inoculation of $10^{3.5}$ egg infectious doses (EID) of mouse-adapted influenza A/Port Chalmers/73 (ref. 12). Target cells were all prepared in a similar manner, as described in the legend of Fig. 1, except that herpes simplex virus type 1, strain Rodanus (HSV-1)¹⁷, target cells were infected with one PFU per cell immediately before chromium labelling, and influenza target cells were BALB/c embryo kidney cells infected with one EID of virus per cell 24 h before labelling.

were eliminated by treatment of spleen cell suspensions with anti- θ serum and complement, but not by removal of adherent cells with nylon wool. Because repeated washing of the suspensions reproducibly increased their cytotoxic activity, it was concluded that an antibody-mediated mechanism was not involved. Other characteristics of CTL induced by murine viral infection, recently reviewed¹⁹, include virus specificity and restriction of target cell recognition to those having the same H-2 haplotype as the effector cells. In our experiments, MCMV immune CTL did not lyse uninfected, HSV-1-infected, or influenza-infected target cells, but the latter could readily be lysed by influenza immune CTL. In addition, when CTL from immune BALB/c (H-2^d) and C3H/HeN (H-2^k) mice were reacted with target cells prepared from the congenic mouse strains B10.D2 (H-2^d) and B10.BR (H-2^k)¹⁸, each lysed only target cells of the same H-2 haplotype. These data thus define the occurrence of a virus-specific, H-2-restricted, cytotoxic T-lymphocyte immune response to MCMV infection.

Several investigators have studied cell mediated cytotoxicity directed against herpesvirus infections, including Epstein Barr virus (EBV)²⁰, HSV-1^{21,22}, and CMV^{16,23-26}. Hutt *et al.*²⁰ reported that unfractionated peripheral blood lymphocytes from patients with acute infectious mononucleosis were capable of lysing lymphoid cell lines carrying EBV. Pfizenmaier *et al.*²¹ found lymphocytes in lymph nodes of HSV-1-infected mice which, when incubated with target cells for six hours, were cytotoxic to virus-infected, but also to uninfected target cells. In addition to the problem of

Table 3 H-2 restriction of target cell recognition by MCMV immune spleen cells

Effector*	Effector	Target	Target	% Lysis
BALB/c	d	d	BALB/c	19.3%†
BALB/c	d	d	B10.D2	10.1%†
BALB/c	d	k	B10.BR	6.5%
C3H/HeN	k	d	B10.D2	3.3%
C3H/HeN	k	k	B10.BR	21.4%†

*Weanling BALB/c and C3H/HeN mice were infected i.p. with 10,000 PFU of MCMV and cell suspensions prepared from spleens removed 14 d later. MEC cultures were prepared from embryos of BALB/c mice and the congenic strains B10.D2 and B10.BR (Jackson)¹⁸, infected with 1-5 PFU per cell of MCMV, depending on the mouse strain of MEC, and chromium labelled. By indirect immunofluorescence it was shown that greater than 80% of target cells were infected.

†Significant difference between test and spontaneous c.p.m. $P < 0.01$; for other results there was no significant difference.

nonspecificity, their data demonstrating abrogation of the effect by pretreatment with anti-Thy 1.2 serum plus complement should be considered as only suggestive evidence that the effectors were T-lymphocytes. In a somewhat different short-incubation assay they did find that cytolysis was specific for target cells which were HSV-1 infected, but they failed to elucidate the cell type responsible for this lysis. Other studies involving human CMV^{16,23-26} and HSV²² have been similarly limited by design or purpose, and have not clearly shown T-cell mediated, virus-specific cytotoxicity. The results reported here, therefore, present the first unequivocal demonstration of such a T-lymphocyte effect in a herpesvirus infection. This CTL response is initiated promptly after infection and may well contribute to the control of primary infection by producing early lysis of antigen-bearing cells.

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Induction of anti-idiotypic antibodies by immunisation with syngeneic spleen cells educated with acetylcholine receptor

THE variable regions of antibodies contain unique antigenic markers called idiotypes^{1,2}. According to Jerne's network theory, antibodies reacting specifically with idiotypic determinants (anti-idiotypes) might be involved in a normal event that modulates the degree and duration of the immune response³. In view of this hypothesis anti-idiotypic antibodies may have a practical application in medicine, for example, in the treatment of autoimmune diseases or other immunological disorders. It has been suggested that the fluctuating clinical symptoms in autoimmune diseases such as myasthenia gravis or systemic

lupus erythematosus are due to a regulatory effect of anti-idiotypic antibodies and that remissions are associated with an increase of specific anti-idiotypic activity⁴. Antibodies and antigen-binding receptors on the surface of lymphocytes share idiotypic determinants^{5,6}. Thus, autoimmunisation with an individual's antibodies or lymphocytes carrying specific receptors for a given antigen may be an efficient way to produce specific anti-idiotypic antibodies⁷⁻⁹. The acetylcholine receptor (AChR) is a major autoantigen in myasthenia gravis and immunisation of animals with AChR (isolated from electric fish) results in the induction of an experimental autoimmune disease (experimental autoimmune myasthenia gravis) similar to myasthenia gravis^{10,11}. We have therefore prepared anti-idiotypic serum specific to AChR idiotypes to test whether it can affect the immune response against AChR and the clinical state resulting from AChR sensitisation. We describe here the preparation of specific C57BL/6J antiserum against AChR-educated lymphocytes and report its anti-idiotypic activity.

Education of lymphocytes was achieved by the procedure of Ishizaka and Adachi¹². A cell suspension from C57BL/6J spleens was cultured for 1 h on tissue culture plates (60 × 10⁶ cells per plate) in minimum essential medium containing 10% fetal calf serum. The nonadherent cells were collected and kept at 4°C. The adherent cells were incubated with purified AChR of *Torpedo californica*¹³ (10 µg per plate) for 1 h. The plates were washed and the previously removed nonadherent cells were added to each plate (40 × 10⁶ cells per plate) in a highly supplemented RPMI-1640 medium. The cells were fed with this medium on day 2 and day 4 and were collected on day 5.

Table 1 Antibody response to AChR of C57BL/6J mice reconstituted with syngeneic AChR-educated lymphocytes

Group	Cells transferred into lethally-irradiated mice	log ₂ haemagglutination titre (day 21)
1	Bone marrow cells (20 × 10 ⁶)	2.7 ± 0.7
2	Bone marrow cells (20 × 10 ⁶) + thymocytes (10 × 10 ⁶)	1.5 ± 1.8
3	Bone marrow cells (20 × 10 ⁶) + thymocytes (100 × 10 ⁶)	6.2 ± 0.9
4	Bone marrow cells (20 × 10 ⁶) + <i>in vitro</i> educated lymphocytes (10 × 10 ⁶)	6.2 ± 1.4

Cell transfer experiments in lethally irradiated mice (800 rad ⁶⁰Co whole-body irradiation) were performed in a procedure similar to that of McDevitt and Tyan¹⁴. AChR (10 µg in complete Freund's adjuvant) was injected to each mouse 24 h after cell transfer.

The specificity of AChR-educated lymphocytes was verified by adoptive transfer (Table 1). Thus the humoral immune response to AChR of lethally irradiated syngeneic mice reconstituted with normal bone marrow cells (20 × 10⁶) and a limited number of educated spleen cells (5-15 × 10⁶) was significantly higher than that of mice reconstituted with bone marrow cells and a similar limited number of normal thymocytes (10 × 10⁶ cells), and was as high as the optimal response obtained in animals reconstituted with 100 × 10⁶ normal thymocytes and bone marrow cells (Table 1).

Anti-idiotypic antibodies were elicited in C57BL/6J mice by repeated intradermal immunisations with 5 × 10⁷ AChR-educated spleen cells per mouse. The first injection was given in complete Freund's adjuvant and successive injections in incomplete Freund's adjuvant. The activity of the resulting antisera was determined by measuring either their direct binding to AChR specific antibodies (Table 2) or their ability to inhibit the binding of ¹²⁵I-AChR to mouse anti-AChR antibodies (Tables 4 and 5).

Direct binding of the anti-idiotypes to anti-AChR antibodies was determined by a solid phase radioimmunoassay¹⁵ in which labelled C3H.SW anti-Ig-1^b (anti-constant region heavy chain

Table 2 Solid-phase radioimmunoassay for direct binding of C57BL/6J anti-idiotypic to various anti-AChR antibodies

Antibodies	Ig-1 allotype	Specific binding (c.p.m.) anti-idiotypic (dilution)		
		1/10	1/100	1/1000
C57BL/6J anti-AChR [(Fab') ₂]	b	1,300	165	ND
AKR/Cu anti-AChR	d	1,400	1,400	700
SWR anti-AChR	c	2,400	480	120
ASW anti-AChR	e	1,140	1,000	ND
C3H.SW anti-AChR	a	1,360	510	ND
BALB/BL anti-NIP-OVA	a	45	0	0
C3H.SW anti-(T,G)-A—L	a	75	30	0
Rabbit anti-AChR		2,100	870	180
Rat anti-AChR		1,800	780	70
Monkey anti-AChR		2,840	725	0

Wells of microtitre plates were coated with 100 µl of the purified antibodies (50 µg ml⁻¹). C57BL/6J anti-idiotypes or, for control, normal C57BL/6J sera in various dilutions were added to the coated wells (25 µl per well) and incubated for 2 h. The plates were then washed twice with 5% foetal calf serum and labelled anti-allotype antibody (25 µl per well, approximately 10⁵ c.p.m.) was added and incubated overnight at 4°C. Finally the plates were washed and the wells were separated and counted. Each dilution was assayed in triplicate and the amount of specific binding was determined after subtraction of the background radioactivity obtained after incubation with normal C57BL/6J serum. The background values were not concentration-dependent and were 200–500 c.p.m. for the different antibodies tested. ND, not determined.

allotypic b markers) antibodies were utilised to measure the specific binding of the C57BL/6J (Ig-1^b) anti-idiotypes to the tested idiotypes. The tested idiotypes were adsorbed to wells of plastic microhaemagglutination plates. The C57BL/6J anti-idiotypic serum or alternatively normal C57BL/6J serum was then reacted with the adsorbed idiotypes and unbound material was removed by extensive washing. Specific binding was determined by further addition of labelled anti-Ig-1^b (Table 2). We found that the C57BL/6J anti-idiotypic preparation bound specifically to the (Fab')₂ fragments of C57BL/6J anti-AChR antibodies. Nevertheless, anti-AChR antibodies of other mouse strains of different genetic background and/or possessing different *H-2* haplotypes or *Ig-1* allotypes also bound to this anti-idiotypic sera (Table 2), demonstrating a broad cross reactivity among variable region determinants of mouse antibodies specific to AChR. Anti-AChR antibodies of other animals, such as rat, rabbit and monkey, react also with the anti-mouse idiotypes (Table 2). The specificity of the anti-idiotypic serum to AChR antibodies was verified by its inability

Table 3 Anti-idiotypic serum does not contain anti-AChR activity

Antiserum tested	Serum dilution	AChR		Monkey anti-AChR antibodies binding
		Antigen binding* (%)	Binding† (c.p.m.)	
C57BL/6J anti-idiotypic	1/10	1	770	3,880
	1/10 ²	0	0	4,780
	1/10 ³		0	3,070
C57BL/6J anti-AChR	1/10	67	41,530	9,180
	1/10 ²	60	49,530	1,170
	1/10 ³	52	50,960	700
	1/10 ⁴	39	41,210	ND
	1/10 ⁵	21	28,100	ND
	1/10 ⁶	8	18,460	ND

*Antigen-binding was assayed by radioimmunoassay using ¹²⁵I-AChR as the labelled antigen and goat anti-mouse Fab to precipitate the antibody-antigen complex.

†Binding assay was performed by solid phase radioimmunoassay as described in Table 2. The wells were coated with AChR and purified monkey anti-AChR antibodies, respectively (50 µg ml⁻¹).

to bind to antibodies against poly(LTyr,LGlu)-poly(DLA1a)-poly(LTyr) [(T,G)-A—L] and the 3-nitro-4-hydroxy-5-iodophenylacetyl conjugate of ovalbumin (NIP-OVA). Table 3 shows that the direct binding assay indeed measures idiotypic-anti-idiotypic binding and not a side reaction between AChR and anti-AChR which could have resulted from the procedure of preparation of purified anti-AChR antibodies and anti-idiotypic serum, respectively. The anti-idiotypic preparation did not contain any appreciable amount of anti-AChR activity, nor did purified anti-AChR contain AChR in amounts that could account for the binding obtained between the anti-idiotypic and the purified antibodies (Table 3).

The activity of the anti-idiotypic antibodies was measured also by their ability to inhibit the binding of ¹²⁵I-AChR to anti-AChR antibodies (the idiotypic). This indirect titration is based on the assumption that the idiotypic determinants or at least part of them may be associated with the antibody-combining site¹⁶. Table 4 shows the degree of inhibition of the binding of ¹²⁵I-AChR to purified C57BL/6J anti-AChR antibodies by the C57BL/6J serum raised against AChR-educated lymphocytes. Furthermore, the binding of ¹²⁵I-AChR to anti-AChR sera of the different mouse strains tested, was also inhibited by the anti-C57BL/6J idiotypes (Table 5). No inhibition of a non-relevant antibody-antigen reaction, such as C57BL/6J anti-(T,G)-A—L and its homologous antigen was obtained by the anti-idiotypic serum, indicating its specificity. These results suggest that mouse AChR idiotypic determinants associated with the antigen-combining site of AChR antibodies are also cross reactive; this is in correlation with the data obtained by the direct binding assay (Table 2), which detects all the possible variable region markers of AChR antibodies against which the anti-idiotypes are directed.

Table 4 Effect of C57BL/6J anti-idiotypic antibodies on the binding of ¹²⁵I-AChR to purified C57BL/6J anti-AChR antibodies

Anti-idiotypic serum (dilution)	Binding (%)
—	100
1:5	20
1:10	25
1:30	85
1:90	90
1:270	97.5

Purified C57BL/6J anti-AChR antibodies (25 ng, purified on a Sepharose-toxin-AChR immunoabsorbent) were incubated with 25 µl of the anti-idiotypic serum dilution (in 5% normal mouse serum) for 15 min at room temperature. ¹²⁵I-AChR (5 ng in 25 µl) was then added and the reaction mixtures were incubated for 30 min at 37°C. Immunoglobulin-bound ¹²⁵I-AChR was co-precipitated by goat anti-mouse Fab serum. The binding in the absence of anti-idiotypic serum was considered at 100%.

Our results show that only immunoglobulin molecules of one distinct antibody specificity (anti-AChR) are recognised by the C57BL/6J serum against AChR-educated syngeneic lymphocytes. Therefore, the determinants carried by the variable region of those immunoglobulin molecules can be defined as idiotypes and the antiserum reacting with them, as anti-idiotypic¹⁸.

The broad cross-reactivity among anti-AChR idiotypic determinants of the specific antibodies of different mouse strains as well as those of different species suggest that similar idiotypic determinants exist in the anti-AChR antibodies tested. Such a situation could be explained if all the tested species inherited a set of *V_H* genes which retained part of the hypervariable region sequence necessary to produce anti-AChR antibodies. This kind of inheritance could be expected in the case of an evolutionary highly conserved antigen such as AChR. Similar hyper-variable regions of antibody combining site in different species

Table 5 Inhibition of the binding of ^{125}I -AChR to anti-AChR sera of different mouse strains by C57BL/6J anti-idiotypic

Anti-AChR serum (mouse strain)	H-2 haplotype	Ig-1 allotype	Inhibition (%)
C57BL/6J	b	b	43.8
CWB	b	b	39.4
AKR/Cu	k	d	21.0
DBA/1	q	c	30.8
SWR	q	c	45.7
ASW	s	e	31.7
SJL	s	b	33.0

Mouse anti-AChR sera were obtained 10 d after two intradermal injections with AChR in complete Freund's adjuvant¹⁷. The inhibition assay was performed as described in Table 3, using 25 μl of 1/5 dilution of the anti-idiotypic serum. The amount of anti-AChR serum used in each case was that which binds 20% of the antigen added. The percentage of inhibition was calculated according to

$$\left(1 - \frac{\text{c.p.m. in the presence of anti-idiotypes}}{\text{c.p.m. in the presence of normal C57BL/6J serum}}\right) \times 100$$

have been demonstrated for the phosphorylcholine system¹⁹. Inheritance of idiotypic determinants have been found also in other antigenic systems in which expression of idiotypic determinants has been shown to be linked to heavy chain allotypic markers²⁰⁻²³.

If anti-idiotypic antibodies play a significant part in controlling autoimmune diseases, their application for treating and managing these diseases should be considered. We have described here our first attempt to prepare anti-idiotypic antibodies specific for an antigen, AChR, known to be actively involved in myasthenia gravis and in the experimental form of this disease. This system may serve as an appropriate tool to study the involvement of anti-idiotypic response in regulating both cellular and humoral immune response to AChR. The broad cross-reactivity among idiotypic determinants in this system is quite encouraging in this respect.

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Differences in secretion of the proteinase cathepsin B at the edges of human breast carcinomas and fibroadenomas

In 1925 Fischer¹ observed that pieces of explanted chicken tumours, unlike normal tissue, caused the lysis of plasma clots. Since then, there have been several investigations into the secretion of degradative enzymes by tumours. An increased extracellular activity of enzymes capable of the destruction of extracellular substances has long been an attractive concept for facilitating the infiltration of normal tissue by tumour cells²⁻⁴. Proteinases are natural candidates for study, as extracellular macromolecules and cell surfaces are rich in proteins and glycoproteins. We report here our study of possible differences in the secretion of the lysosomal proteinases, cathepsin D and cathepsin B, from explants of human tumours, both malignant (breast carcinomas) and non-malignant (breast fibroadenomas) and from normal breast tissue, as previously suggested⁵. Cathepsin B secretion was significantly higher for malignant than non-malignant tissue (fibroadenomas and normal breast tissue). Such a difference in the secretion and extracellular activity of this enzyme might help to explain further the ability of malignant tumours to infiltrate and metastasise.

Breast tissue and non-necrotic tissue removed from the edges of tumours at surgery was immediately cut into small slices approximately 3-5 mm square and no more than 2 mm thick. In the case of operating theatres some distance from the laboratory, tissues were established in organ culture in the theatre in medium BGJw (ref. 10) and transported to the laboratory within three hours in a portable incubator at 37 °C. This method has been successfully used in previous studies of human tissues¹¹. All tumour samples contained some contiguous normal tissue. Breast tissue was also removed remote from tumours at mastectomy and explants were similarly prepared. In the laboratory, all tissues were first washed in medium BGJw before being explanted onto expanded metal grids in Petri dishes as previously described¹². The culture medium was Eagle's DMEM (Wellcome) and contained 5% (v/v) acid-treated sheep serum, heat inactivated at 56 °C for 30 min. Serum was acid treated¹³ to inactivate α_2 -macroglobulin, the major natural inhibitor of cathepsin B in serum¹⁴. Cultures were incubated at 37 °C in a gas phase of 95% air and 5% carbon dioxide. Culture medium was removed every two days, immediately frozen and stored at -20 °C and replaced with fresh medium. For studies of enzyme content, cultured tissues were homogenised in distilled water containing 0.1% (v/v) Triton X-100 (Sigma) with an Ultraturrax homogeniser (Janke and Kunkel). DNA was assayed according to the method of Bonting and Jones¹⁵.

Culture media were assayed for lactate dehydrogenase as described previously¹⁶. Cathepsin D was assayed at pH 3.7 with acid-denatured haemoglobin as a substrate, using a method based on that described by Poole¹⁷. The enzyme was

Table 1 Activities of cathepsin D and cathepsin B in tissue after culture for six days

Enzyme	Carcinomas	Fibroadenomas	Breast tissue
Cathepsin D	10.72 $\pm$ 3.84(8)	9.28 $\pm$ 2.72(5)	7.04 $\pm$ 1.44(6)
Cathepsin B	1.44 $\pm$ 0.48(4)	2.08 $\pm$ 0.80(5)	1.28 $\pm$ 0.64(4)

Cathepsin D activity in nmol tyrosine equivalents produced per μg tissue DNA per 16 h; cathepsin B activity in nmol BANA hydrolysed per μg tissue DNA per 16 h. Mean $\pm$ s.e.m. of number (in parentheses) of different explants examined.

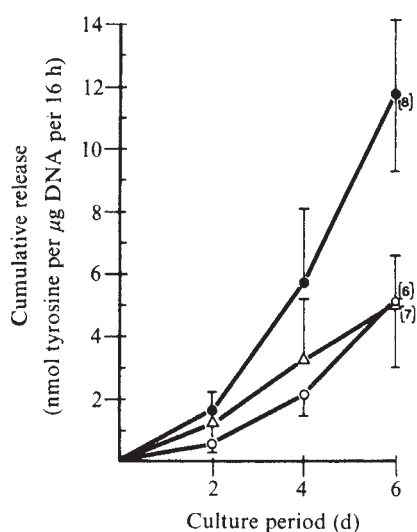


Fig. 1 Accumulation of cathepsin D in culture media of explants from human carcinomas (●), fibroadenomas (○) and normal breast tissue (△). Results show the mean accumulated activity $\pm$ s.e.m. with the number of explants used for each determination given in brackets. The amount of cathepsin D detected in culture media from carcinoma explants is not significantly larger than that from fibroadenomas and normal tissue ($P > 0.1$).

inhibited by pepstatin, an inhibitor of cathepsin D¹⁸. The thiol proteinase cathepsin B was assayed using α -N-benzoyl-D,L-arginine-2-naphthylamide hydrochloride (BANA) as substrate¹⁹. The measured activity required cysteine as activator and was inhibited by 1 mM 4-chloromercuribenzoate, indicating a thiol proteinase. It was also maximally inhibited (90%) by 2.5 μ M leupeptin, a potent inhibitor at this concentration of cathepsins B (ref. 20) and L (ref. 21). However, cathepsin L has no significant activity against BANA²². Hence, the activity is considered to be due mainly to cathepsin B (EC 3.4.22.1). Statistical analyses were carried out using Wilcoxon's rank sum test to a significance level of 5% (ref. 23).

In view of its lability, lactate dehydrogenase (LDH) in culture media was determined first to prevent loss of activity due to repeated freezing/thawing. This enzyme was assayed to determine viability of the tissue, as LDH is normally only released from dead or dying cells^{24,25}. We observed an initial release of LDH which is due to tissue damage incurred in preparing the explants for culture. In the results presented here LDH was not detected after the first medium change. Thus, subsequent release of proteinases from tissue into culture media was considered to result from an active secretion as has been concluded previously (for example, see refs 24, 25).

The total activities of cathepsin D and B in cultured explants of carcinomas, fibroadenomas and breast tissues were examined and found to be similar (Table 1). Figures 1 and 2 demonstrate the comparative accumulation of cathepsin D and B, respectively, in culture media from explants of these tissues. There was no significant difference between the release of cathepsin D from tissues removed from these tumours and from breast tissue. Cathepsin B was detected in comparable amounts per tissue DNA in media from cultures of fibroadenomas and breast tissue. However, in culture media of explants from carcinomas higher levels of cathepsin B were detected. The mean accumulation of cathepsin B activity in the culture medium from carcinomas over a period of 10 d was 7.32 nmol BANA hydrolysed per μ g DNA per 16 h, with a range of 2.41–16.55. For fibroadenomas a mean value of 0.65, with a range of 0.11–2.08

was observed over the same time period, and normal tissue showed a mean accumulation of 0.08 nmol BANA hydrolysed per μ g DNA per 16 h, with a range of 0–0.175 over six days of culture.

Further evidence that this release of proteinases is an active secretion and dependent on protein biosynthesis was obtained by adding cycloheximide to the culture media (10 μ g ml⁻¹). The release of both proteinases was completely inhibited; this would not be the case if these enzymes were leaking out of dying tissue or from extracellular sites where they might have already been present. No evidence could be found for the presence of inhibitors of cathepsin B in culture media which might have been in part responsible for the differences in accumulation of cathepsin B in culture media from carcinomas compared with other tissues.

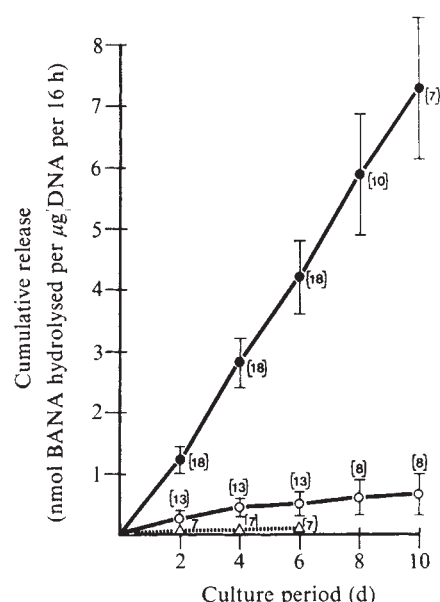


Fig. 2 Accumulation of cathepsin B in culture media of explants from human carcinomas (●), fibroadenomas (○) and normal breast tissue (△). Results show the mean accumulated activity $\pm$ s.e.m. for the number of explants indicated in brackets. The amount of cathepsin B detected in culture is significantly greater than that from fibroadenomas and normal breast tissue ($P < 0.01$).

The precise factors which enable tumour cells to escape normal tissue constraints, to detach from each other and to penetrate physical barriers and metastasise remain obscure. However, it is well known that proteinases can cause detachment of cells from each other and in some cases can initiate cellular division in cultures rendered quiescent by serum deprivation^{26–28}. The preferential secretion by malignant breast tumours of a proteinase such as cathepsin B is of interest, for this enzyme possesses significant activity at pH 7 (refs 29, 30) and it can degrade both collagen³⁰ and proteoglycan³¹, two of the major constituents of extracellular substance. More important, perhaps, this enzyme has been shown to cause cellular detachment from glass at pH 7.1 (ref. 32) indicating that, like trypsin, it has the ability to degrade pericellular protein on or close to cell surfaces. Thus, the activity of cathepsin B could favour a loss of normal tissue constraints to cell movement, leading to metastasis. In our work the cellular source of this enzyme is not yet known. Its extracellular presence, however, and its properties focus attention on its potential importance as a mediator of tumour dissemination.

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Opiate-like and abstinence-like effects of intracerebral histamine administration in rats

HISTAMINE is generally thought to act as a neurotransmitter in the central nervous system (see, for example, ref. 1). Electrophysiological² and biochemical³ studies have indicated that both H₁- and H₂-receptors are present in the brain. Functionally, histamine has been implicated in central mechanisms of thirst⁴ and thermoregulation⁵. A role for

brain histamine in tolerance and physical dependence to morphine has also been suggested^{6,7}. We describe here studies in which we microinjected histamine into various regions of the rat brain and observed analgesia and catalepsy from periaqueductal sites and effects resembling symptoms of morphine withdrawal from sites in the hippocampus.

Female Sprague-Dawley rats, approximately 90 d old, were implanted with intracerebral cannulae (26-gauge) in one of several regions in either the brainstem or forebrain. Except for midline sites (for example, raphe nuclei), cannulae were placed bilaterally and symmetrically. Each rat had cannulae in only one site and was used only once for microinjection testing. Rats were allowed 4–5 d to recover from surgery and standard microinjection procedures were used⁸.

Histamine dihydrochloride, dissolved in physiological saline, was administered in a volume of 0.5–1.0 μ l over 30–60 s. Behavioural testing began 15 min before drug administration and continued for a least two hours afterwards. Response to pin prick and heat-induced tail flick⁹ were used as indices of pain. For the former, an aesthesiometer (Rowan), calibrated at 14 g of pressure, was uniformly applied at nine points on the rat's body (anterior, middle and posterior parts of both sides and back). Responses were recorded as 0 (no response), 1 (local twitch at point of application), 2 (whole body flinch), 3 (squeak) and 4 (excessive squeaking and/or jumping with attempt to bite needle). The presence or absence of catalepsy was determined by observing whether a rat would remain on its back for at least 15 s after being positioned by the experimenter. The occurrences of other spontaneous behaviours, to be described later, were also recorded.

When injected into the dorsal raphe nucleus, or nearby in the periaqueductal grey region, histamine elicited analgesia. Injections in the vicinity of the median raphe nucleus elicited hyperalgesia (Fig. 1). Both the analgesia and the hyperalgesia were apparent within 3–5 min and persisted for 20–60 min, depending on dose (Table 1). Grooming, at low doses (12.5–25 μ g), and catalepsy, at high doses (100–200 μ g), were also observed after injection of histamine into either site. None of these effects occurred after injections of noradrenaline hydrochloride (25–100 μ g) or serotonin hydrochloride (25–100 μ g) into the same regions. Injections of histamine into the locus coeruleus and substantia nigra were without effect. Intraventricular administration of histamine (200–400 μ g) elicited analgesia, catalepsy and hypothermia. Hypothermia alone was observed after administration of histamine to the rostral hypothalamus but not to the lateral or posterior hypothalamus.

The most interesting effects of histamine were observed after injections into the dorsal hippocampus. Beginning within 20–100 min after injection, rats had frequent episodes of teeth chattering, facial tremors, head shakes, 'wet dog shakes', writhing, salivation, yawning, chewing, grooming and irritability (squeaking, jumping and biting in response to handling). Depending on the dose, this syndrome persisted for 2–5 h (Table 2). Only occasional teeth chattering and head shakes occurred after ventral hippocampal injections (Fig. 2). None of these effects was seen after injections into the cortex or corpus callosum above the hippocampus or into the lateral ventricle, lateral thalamus or superior colliculus below the hippocampus. Medial thalamic, medial septal and striatal injections were also without effect. No effects were observed after injections of serotonin (50–200 μ g) and noradrenaline (50–200 μ g) into the dorsal hippocampus.

Hypothermia¹⁰ and catalepsy¹¹ have previously been observed after intracerebral administration of histamine. The present data confirm the involvement of the rostral

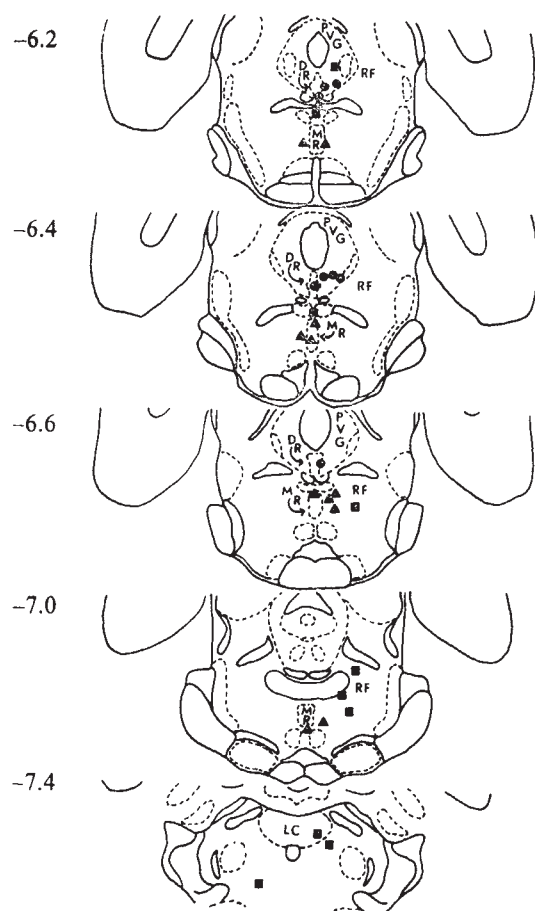


Fig. 1 Brainstem sites where histamine (25–100 μ g) elicited analgesia (●) or hyperalgesia (▲). Also shown are neighbouring sites in which similar microinjections had no effect (■) on pain responses within 60 min. Abbreviations: DR, dorsal raphe nucleus; MR, median raphe nucleus; RF, reticular formation; PVG, periaqueductal-periventricular grey; LC, locus coeruleus. The numbers shown indicate distance (mm) posterior to bregma at which the sections shown are located (in accordance with ref. 30).

hypothalamus in hypothermia and implicate the raphe nuclei as the site mediating the catalepsy. Although catalepsy may ultimately be due to blockade of dopaminergic transmission in the striatum¹², it can possibly be induced indirectly by an action on the raphe nuclei (for example, ref. 13). Certainly, the negative effects of intrastriatal and intranigral histamine would seem to preclude any direct action on nigrostriatal neurones.

The localisation of the histamine-induced analgesia and hyperalgesia to the dorsal raphe-periaqueductal grey and median raphe nucleus, respectively, is of considerable interest, as it is these areas which have been most commonly invoked in discussions of pain mechanisms within the brainstem¹⁴. Opiates^{15–16}, endogenous opiate-like peptides¹⁷ and electrical stimulation¹⁸ induce analgesia when applied to these regions, although hyperalgesia¹⁹ has also been reported. Contradictory results (for example, ref. 20) of changes in morphine analgesia after raphe lesions may, perhaps, be attributable to variable degrees of damage to closely related regions (such as the dorsal and median raphe nuclei) with opposing influences on pain sensitivity.

The delayed appearance of effects following intrahippocampal administration of histamine is quite puzzling. It is unlikely that this delay represents the time necessary for the drug to diffuse and reach a threshold concentration in a critical volume of tissue, as there were no significant

differences in time of onset among doses nor between animals injected with the same doses in different volumes (0.5 compared with 1.0 μ l). It is conceivable that the final action of histamine, in this instance, might be mediated by a metabolite, or possibly by a histamine-containing peptide²¹. However, we have recently observed that dimaprit (50 μ g), a specific H_2 -agonist²², induces the same time-dependent effects as histamine when injected into the hippocampus. This fact makes it unlikely that histamine's effects are mediated by a metabolite. It is also of interest that a histamine-induced release of catecholamines from brain slices similarly develops slowly²³.

Preliminary data indicate that H_1 - and H_2 -receptors are apparently differentially and selectively involved in different effects of histamine. Dimaprit will mimic the effect of histamine in the hippocampus, as already noted, and induce analgesia but not grooming or catalepsy when injected (25–100 μ g) into the dorsal raphe area. Conversely, cimetidine (50 μ g), a specific H_2 -antagonist, blocked the hippocampal symptoms and the dorsal raphe analgesia, respectively, when injected into the same sites as histamine (50–100 μ g). Mepyramine, an H_1 -antagonist, reduced the catalepsy but had no effect on the histamine-induced analgesia and hippocampal symptoms. Further studies with other antagonists are required to substantiate these results.

The histamine-induced hippocampal symptoms were remarkably similar to an opiate abstinence syndrome, whereas the analgesia and catalepsy resulting from brainstem injections seemed analogous to acute opiate effects. Morphine is known to release histamine in brain²⁴ and cessation of chronic morphine administration has been shown to produce a large depletion of brain histamine⁶. Both histamine agonists and antagonists have been found to ameliorate the expression of physical dependence on morphine withdrawal⁷. Taken together, these findings suggest that, in a complex way, histamine is involved in opiate dependence. If opiates release histamine from both the brainstem and hippocampus, it seems that the effect in the brainstem should predominate, as intraventricular administration of histamine mimicked the effect of brainstem rather than of hippocampal injection. As a histaminergic pathway ascending in the medial forebrain bundle to the hippocampus has been described²⁵, perhaps histamine itself inhibits this pathway by acting on cell bodies and/or dendrites in the brainstem, similarly to other self-regulatory mechanisms proposed for dopaminergic²⁶ and serotonergic²⁷ pathways. On withdrawal of chronic opiate administration, the ascending histaminergic pathway might exhibit compensatory hyperactivity and release excessive amounts

Table 1 Analgesic-hyperalgesic effects of brainstem histamine injections

Site	n	Dose (μ g)	Mean pain sensitivity scores(s)*	
			Pin-prick	Tail-flick
Dorsal raphe	16	0 (pre-injection)	1.9	1.0
	2	0 (saline)	2.2	1.0
	5	6.25–12.5	1.2†	2.0†
	5	25–50	1.1†	4.2†
	4	100	0.6†	5.7†
Median raphe	19	0 (pre-injection)	1.8	1.0
	2	0 (saline)	2.2	1.0
	4	6.25–12.5	3.0†	1.0
	7	25–50	3.5†	1.0
	6	100	3.7†	1.0

*Scores shown are those recorded 10 min pre- and post-injection. At both sites, duration of effect varied directly with dose.

†Significant from pre-injection values at $P < 0.05$ – 0.02 , Wilcoxon signed rank test.

Table 2 Time-course of effects of dorsal hippocampal histamine injections

Dose (μ g)	-10 min	5 min	20 min	Mean total test score*		90 min	120 min	180 min	300 min
0 (saline) (n = 3)	0.7	0	0.3	0	0	0	0	0	0.3
6.25-25 (n = 5)	0.4	0.2	1.2†	1.2†	1.4†	1.8†	2.0†	0.8	0
50 (n = 8)	0.8	0.6	2.9†	3.6†	4.5†	5.1†	7.8†	6.5†	1.3
100 (n = 7)	0.7	1.1	3.7†	5.3†	6.8†	7.9†	9.4†	8.0†	3.4†

*At each time interval after or before (-10 min) injection, the presence or absence of 10 effects was noted and scored (0 = absent, 1 = present; maximum total score = 10) during a five-minute observation period. The 10 effects were: teeth chattering, facial tremors, head shakes, 'wet dog shakes', writhing, salivation, yawning, chewing, grooming and irritability. Although the test scores do not reflect the intensity of any individual effect, it should be noted that the frequency of 'wet dog shakes' ranged as high as 10 per min.

†Significant from pre-injection values at $P < 0.05-0.001$, Wilcoxon signed rank sum test.

of histamine in the hippocampus; hence the similarity of intrahippocampal histamine injections to opiate abstinence.

Methionine-enkephalin and morphine have been reported to elicit electrographic seizures and 'wet dog shakes' in rats when administered into or dorsal to the lateral ventricle²⁸. Naloxone failed to prevent or reverse enkephalin-induced seizures, suggesting that the epileptogenic action was indirectly mediated by a non-opiate receptor. Data from

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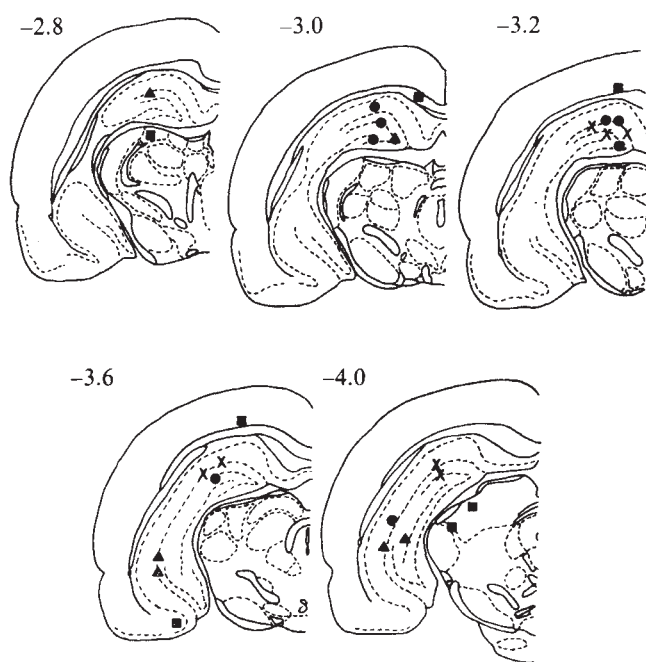


Fig. 2 Hippocampal sites where histamine (50-100 μ g) elicited, within 2 h of administration, teeth chattering (▲), teeth chattering plus wet dog shakes (●) or teeth chattering, wet dog shakes and salivation (×). Also shown are neighbouring sites in which similar microinjections had no effect (■).

other laboratories indicated that 'wet dog shakes' reflect paroxysmal activity in the hippocampus, regardless of whether such activity is induced by electrical or chemical means²⁹. Thus, a local action in the hippocampus involving histamine release may underlie the seemingly paradoxical occurrence of opiate-elicited 'wet dog shakes'. To the extent that specific histamine receptors can be shown to be involved in specific aspects of opiate abstinence, our results may have significant therapeutic implications.

Amide and l-amino derivatives of F prostaglandins as prostaglandin antagonists

THE formation in tissues and organs of the different prostaglandins (PGs) is now known to be associated with various pathophysiological situations. There is an urgent need for specific PG antagonists to facilitate experimental differentiation and analysis of the roles of these endogenous PGs. Compounds which have been introduced as PG antagonists include 7-oxaprostanolide analogues (for example, 7-oxa-13-prostynoic

acid)¹, dibenzoxazepine derivatives (SC-19220)², and phosphorylated polymers of phloretin (PPP)³. More recently, sodium-4-(1-oxo-2-(4-chlorobenzyl)-3-phenylpropyl)phenylbenzyl phosphonate (N-0164)⁴ and 8-ethoxycarbonyl-10,11-dihydro A prostaglandins⁵ have been studied. Unfortunately, although these compounds inhibit *in vitro*, their usefulness is limited in terms of potency, specificity and competitiveness, especially in the intact animal. We describe here a simple and novel approach to the design of PG antagonists which are active both *in vitro* and *in vivo*⁶.

The various PG derivatives were synthesised from either PGF_{2α}, 15-*epi*-PGF_{2α}, 5-*trans*-PGF_{2α}, or PGF_{1α}, and were isolated in very high purity (with rigorous exclusion of the biologically active starting materials) by careful chromatographic separation. Treatment of PGF_{2α} methyl ester (34 mg) with ammonium chloride (130 mg) and 1 ml of liquid ammonia in a sealed tube at 65 °C for 36 h afforded essentially pure primary amide (I in Fig. 1) (32 mg, 93% yield) as a colourless oil: {α}_D²⁵+36.7° (c 1.5, methanol) (*R*_f 0.37, 20% methanol-methylene chloride). This material was purified by preparative thin layer chromatography (ptlc) (Merck 20×20×0.025 cm glass-backed silica gel plates) (20% methanol-methylene chloride, one development). Exposure of PGF_{2α} methyl ester (83 mg) to methanolic hydroxylamine (15 equiv.) and sodium hydroxide (8 equiv.) at 25 °C for 18 h (ref. 7), followed by work-up and ptlc (benzene-dioxane-acetic acid 10:10:1, two developments) afforded pure hydroxamic acid II as a viscous oil (67 mg, 80% yield): {α}_D²⁵+31.4° (c 2.7, methanol) (*R*_f 0.10, benzene-tetrahydrofuran-acetic acid 20:20:1, two developments). Reaction of PGF_{2α} methyl ester (21.4 mg) with methylamine hydrochloride (40 mg) and methylamine (1.5 ml) in a sealed tube at 75 °C for 10 h furnished essentially pure monomethyl amide III (Fig. 1) (99% yield). The product was subjected to ptlc (benzene-tetrahydrofuran-acetic acid 20:20:1, four developments), affording pure III as a colourless oil: {α}_D²⁵+32.9° (c 1.78, methanol) (*R*_f 0.48, 20% methanol-methylene chloride). PGF_{2α} dimethyl amide (IV) was prepared by heating a solution of PGF_{2α} methyl ester (36.7 mg) and dimethylamine hydrochloride (60 mg) in 1.5 ml of dimethylamine at 75 °C for 36 h in a sealed tube. Work-up followed by ptlc (benzene-tetrahydrofuran-acetic acid 20:20:1, three developments) afforded pure IV as a pale yellow oil (36.6 mg, 96% yield): {α}_D²⁵+27.2° (c 3.6, methanol) (*R*_f 0.23, 10% methanol-methylene chloride). Following the same procedure used for IV, 5-*trans*-PGF_{2α} dimethyl amide (V) was obtained from the corresponding methyl ester as an oily solid: {α}_D²⁵+8.7° (c 0.23, chloroform) (*R*_f 0.22, 10% methanol-methylene chloride). Reduction of the primary amide of PGF_{2α} (I) with ethereal lithium aluminium hydride (6 mol equiv.) in tetrahydrofuran at 25 °C for 36 h furnished the primary amine VI. After work-up the crude product was dissolved in dilute aqueous acid, the solution was washed with ether to remove non-basic materials, and the amine was regenerated with base and isolated, affording pure VI as an oil: {α}_D²⁵+29.5° (c 1.1, methanol) (*R*_f 0.11, methanol). The dimethyl amide of PGF_{2α} (IV) was reduced according to the procedure described for VI, affording the dimethyl amine VII (96% yield) as a colourless oil: {α}_D²⁵+32.3° (c 1.5 methanol) (*R*_f 0.23, methanol). Reduction of PGF_{2α} methyl ester (25.0 mg) with diisobutylaluminium hydride (6 equiv.) in ether at 25 °C for 8 h afforded nearly pure primary alcohol VIII (23.0 mg, 99% yield) as a colourless oil. Trace impurities were removed by ptlc (20% methanol-methylene chloride, two developments). The purified product had {α}_D²⁵+25.1° (c 1.5, methanol) (*R*_f 0.16, 10% methanol-methylene chloride). The primary amide of 15-*epi*-PGF_{2α} (X) was prepared from the corresponding methyl ester by the procedure described for I. Ptlc (10% methanol-methylene chloride, four developments) furnished X as a colourless oil: {α}_D²⁵+14.4° (c 0.34, chloroform) (*R*_f 0.49, 20% methanol-methylene chloride). Following the procedure described for III, 15-*epi*-PGF_{2α} methyl ester was converted to the monomethyl amide XI. Ptlc (10% methanol-methylene chlor-

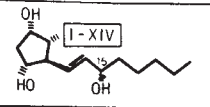
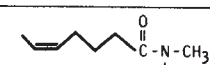
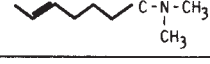
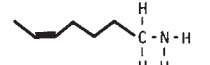
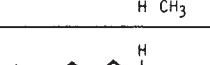
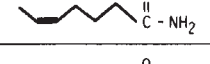
		15-OH	Relative potency (PGF _{2α} = 100)	Antagonism to PGF _{2α} (% Block)*
I		α	0.3	0
II		α	NT	0
III		α	< 0.3	0
IV		α	< 0.3	50
V		α	< 0.3	25
VI		α	NT	0
VII		α	< 0.3	60
VIII		α	0.6	0
IX		β	50	100
X		β	< 0.3	0
XI		β	< 0.3	0
XII		β	< 0.3	55
XIII		α	NT	50
XIV		α	NT	45

Fig. 1 Antagonistic activity and relative potency of PGF derivatives on gerbil colon. *, % Inhibition at 3.2 μg ml⁻¹. NT, not tested.

ide, three developments) afforded XI as a colourless oil: {α}_D²⁵+22.6° (c 0.39, chloroform) (*R*_f 0.17, 10% methanol-methylene chloride). The dimethyl amide of 15-*epi*-PGF_{2α} (XII) was obtained from the corresponding methyl ester using the procedure described for IV. After ptlc (benzene-tetrahydrofuran-acetic acid 20:20:1, two developments) XII was isolated as a colourless oil (*R*_f 0.24, benzene-dioxane-acetic acid 20:20:1). Following the procedure described for IV, PGF_{1α} methyl ester was converted to the dimethyl amide XIII. Ptlc (benzene-tetrahydrofuran-acetic acid 20:20:1, four developments) afforded XIII as a solid: m.p. 95–97.5 °C; {α}_D²⁵+19.7° (c 0.85, methylene chloride) (*R*_f 0.54, 20% methanol-methylene chloride). The reduction of PGF_{1α} dimethyl amide (XIII)

as described for VII furnished the oily dimethyl amine XIV: $\{\alpha\}_D^{25} + 15.4^\circ$ (c 0.65, chloroform) (R_f 0.13, methanol). All structural assignments are consistent with spectral data.

The preliminary testing of the antagonists described here was performed on the gerbil (*Meriones unguiculatus*) colon. The ascending colon from gerbils weighing 60–80 g was suspended in a tissue bath (2 ml) containing de Jalon's solution at 30 °C which was equilibrated with room air as described by Weeks⁸. Isotonic contractions were recorded with a rotary motion transducer (Harvard Model 382). After the tissue was allowed to stabilise, a dose-response curve to $\text{PGF}_{2\alpha}$ or PGE_2 was established. The dose range for PGE_2 was 0.5 ng ml⁻¹ to 8 ng ml⁻¹ and that for $\text{PGF}_{2\alpha}$ was 4 ng ml⁻¹ to 32 ng ml⁻¹. Dose-response curves for acetylcholine, (8–32 ng ml⁻¹), bradykinin, (0.5–2 ng ml⁻¹) and for those derivatives which exhibited agonist potency were also determined. The tissue was exposed to a compound for 0.75 min. Relaxation was obtained by washing with de Jalon's (7 ml min⁻¹ for 1 min followed by 3.5 ml min⁻¹ for 2.75 min), and then a rest period of 0.5 min elapsed before injecting the next dose. For testing antagonism, a dose-response curve to the PG and acetylcholine was established. The derivatives were then perfused for 12–15 min, and the dose-response curves to $\text{PGF}_{2\alpha}$ or PGE_2 and acetylcholine were repeated. In most cases, the curve returned to the initial values after a 15-min wash with de Jalon's solution. Initially, the antagonism was measured at several concentrations of each derivative, but later one concentration (3.2 $\mu\text{g ml}^{-1}$) was used arbitrarily for all the compounds to conserve the stock of antagonists. The potency ratio of each compound relative to $\text{PGF}_{2\alpha}$ and the percentage antagonism to $\text{PGF}_{2\alpha}$ were determined from the log dose-response curve at the approximate⁵⁰ ID₅₀ level for a $\text{PGF}_{2\alpha}$ response (dose required for 50% maximal contraction). Those PGs used for bioassay were prepared as stock solutions (1 mg ml⁻¹) in 95% ethanol and diluted before use with de Jalon's solution so that volumes added to the muscle bath did not exceed 64 μl . Acetylcholine chloride (Sigma) and Lys-bradykinin (Beckmann) were prepared as 1 $\mu\text{g ml}^{-1}$ solutions in de Jalon's on the day of the experiment. The stock solutions of the derivatives were prepared in 95% ethanol and diluted with de Jalon's before use.

The effects of 14 derivatives on the response of the gerbil colon to $\text{PGF}_{2\alpha}$ are shown in Fig. 1. Each compound was tested between three and eight times. The greatest antagonism to $\text{PGF}_{2\alpha}$ was achieved with the dimethyl amides and dimethylamino compounds. At a concentration of 3.2 $\mu\text{g ml}^{-1}$, these compounds inhibited by 25–60% the contraction induced by $\text{PGF}_{2\alpha}$ at its approximate ID₅₀ level (6 ng ml⁻¹). The dimethyl amides of $\text{PGF}_{2\alpha}$ (IV), 15-*epi*- $\text{PGF}_{2\alpha}$ (XII), and $\text{PGF}_{1\alpha}$ (XIII), as well as the dimethylamino compounds VII and XIV, all displayed 45–60% inhibition, while the 5-*trans*- $\text{PGF}_{2\alpha}$ dimethyl amide (V) was about half as effective. At this concentration (equivalent to $8.7 \times 10^{-6}\text{M}$) the most active derivative was the dimethylamino compound (VII) which antagonised the response to $\text{PGF}_{2\alpha}$ by 60%; the dose ratio in the presence and absence of VII was 2.6 ± 0.5 (s.e.m.). The $\text{PGF}_{2\alpha}$ dose-response curve was displaced in a manner indicating dose-dependent competitive inhibition by both the dimethylamino (VII) and dimethyl amide (IV) derivatives (Fig. 2). No antagonism to acetylcholine- or bradykinin-induced contractions was observed with either derivative at this concentration; PGE_2 contractions were also antagonised (Fig. 3) but not as much as $\text{PGF}_{2\alpha}$; the dose ratio was 1.5 ± 0.2 (s.e.m.). More importantly, no agonist behaviour was exhibited with any of the dimethylamino or dimethyl amide derivatives at concentrations up to 40 times the approximate ID₅₀ value. In other cases, 15-*epi*- $\text{PGF}_{2\alpha}$ (IX) and the 1-hydroxy derivative of $\text{PGF}_{2\alpha}$ (VIII) possessed some agonist activity. The $\text{PGF}_{1\alpha}$ derivatives XIII and XIV were tested for antagonism both to $\text{PGF}_{1\alpha}$ and also to $\text{PGF}_{2\alpha}$. Each of these compounds antagonised both $\text{PGF}_{1\alpha}$ and $\text{PGF}_{2\alpha}$ with comparable effectiveness and consequently, specificity as to the degree of unsaturation was not present.

The ID₅₀ values for the PG antagonists described in the

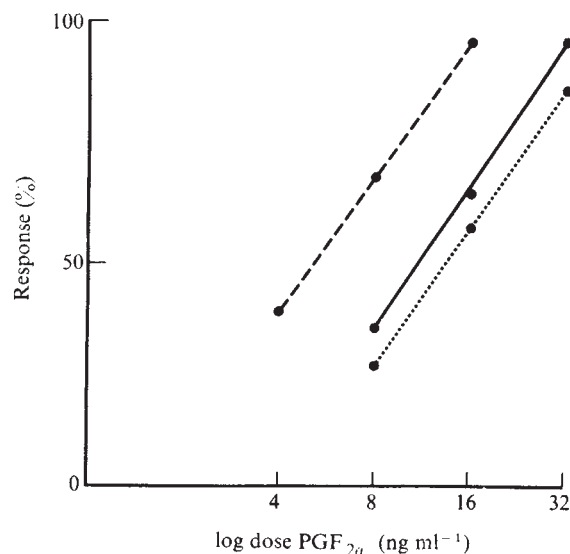
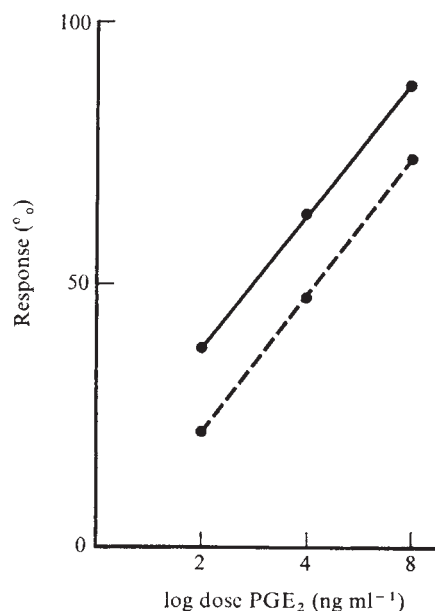


Fig. 2 Three-point log dose-response curves for $\text{PGF}_{2\alpha}$ alone (---) and in the presence of 3.2 $\mu\text{g ml}^{-1}$ $\text{PGF}_{2\alpha}$ dimethyl amide (IV) (—) and $\text{PGF}_{2\alpha}$ dimethylamine (VII) (···). Each point is the average of determinations made on six gerbil colon preparations. The difference in potency between the two antagonists was not significant. No antagonism of equi-active doses of either acetylcholine or bradykinin was observed.

literature vary from $4.4 \times 10^{-7}\text{M}$ for 8-ethoxycarbonyl-10,11-dihydro A prostaglandins⁵ to $7.6 \times 10^{-5}\text{M}$ for 7-oxa-13-prostynoic acid derivatives¹. The ID₅₀ of the dimethyl amide and dimethylamino derivatives of $\text{PGF}_{2\alpha}$ falls in the middle of this range.

The configuration at C-15 seems not to be critical since the dimethyl amide derivatives of $\text{PGF}_{2\alpha}$ (IV) and 15-*epi*- $\text{PGF}_{2\alpha}$ (XII) had the same antagonist activity. The recent observation⁹ that various 11,15-bisdeoxy PGE_1 derivatives, including C-1 amide derivatives, antagonise PGE_1 is complementary to our own findings.

Fig. 3 Three-point log dose-response curves for PGE_2 alone (—) and in the presence of 3.2 $\mu\text{g ml}^{-1}$ of $\text{PGF}_{2\alpha}$ dimethylamine (VII) (---). Each point is the average of determinations made on three gerbil colon preparations.



These results¹⁰ demonstrate a novel but simple approach to the design of PG antagonists which are structurally related to natural biologically active PGs. The antagonism by both the dimethyl amide and dimethylamino derivatives (IV and VII) of the pressor response to PGF_{2α} in the perfused canine pulmonary lobe has been described elsewhere⁶.

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Inhibition of prostaglandin biosynthesis by corticosteroids requires RNA and protein synthesis

THE finding that aspirin-like drugs act by inhibiting prostaglandin (PG) synthetase was reported by Vane *et al.*¹ and the possible effects of anti-inflammatory steroids on PG production have since been a matter of controversy. Although some studies have indicated no appreciable effect of the corticosteroids on PG synthetase^{1–4}, other investigators have presented evidence that corticosteroids may act by inhibiting the biosynthesis or release of PGs. The latter data were derived from studies on a variety of tissues such as skin⁵, adipose tissue⁶, inflamed synovia^{6,7}, cultured fibroblasts⁸ and fibrosarcoma cells⁹. No satisfactory explanation for these controversial results has been offered, nor has the mechanism of this possible effect of corticosteroids on PG production been properly elucidated. The mechanism of action of the steroid hormones on their target organs has been clarified in recent years. These hormones are now thought to act primarily by stimulating transcription, thus controlling the rate of synthesis of certain key proteins (see ref. 10 for review). Many of the actions of the corticosteroids on intermediary metabolism are indeed known to involve the induction of synthesis of specific enzyme proteins. We report here that corticosteroids influence PG production by a mechanism involving RNA and protein synthesis.

Renal papillae from female Charles-River rats (weighing 150–200 g) were minced and placed in 2 ml of Dulbecco's modified Eagle's medium (DMEM), pH 7.4. Incubation was carried out in a metabolic shaker at 37 °C, and the medium

was replaced every 30 min. PGs of the E or F groups were determined by radioimmunoassay, using antisera to PGE-bovine serum albumin (BSA) and PGF-BSA, respectively, and the procedures of Miles-Yeda. Cortisol (10⁻⁶ M, as hemisuccinate) produced greater than 50% inhibition of PGE output, after an initial latency of 60 min (Fig. 1). The addition of either actinomycin D (0.5 µg ml⁻¹) or cycloheximide (1 µg ml⁻¹) abolished the inhibitory effect of cortisol. These two antibiotics are known inhibitors of

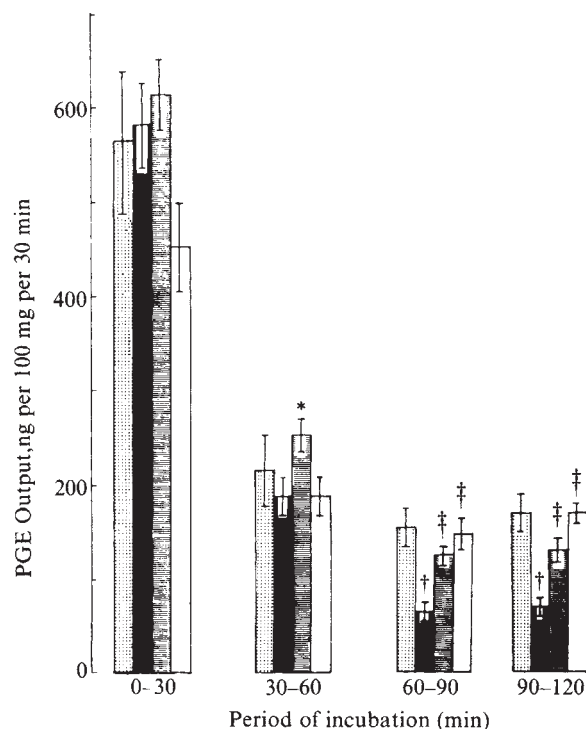


Fig. 1 Time course of PGE output from rat renal papilla and the effect of cortisol in the absence or presence of inhibitors of RNA and protein synthesis. DMEM containing the drugs specified was replaced every 30 min and PGE was determined by radioimmunoassay. Dotted column indicates control incubations; solid column indicates cortisol (hemisuccinate) 10⁻⁶ M; lined column indicates cortisol 10⁻⁶ M + actinomycin D (0.5 µg ml⁻¹); open column indicates cortisol 10⁻⁶ M + cycloheximide (1 µg ml⁻¹). Bars represent means ± s.e.m. of six experiments.

*Significant differences compared to cortisol (Student's *t*-test) $P < 0.05$.

†Significant differences compared to control, $P < 0.005$.

‡Significant differences compared to cortisol, $P < 0.005$.

DNA-dependent RNA synthesis and protein synthesis respectively. Table 1 shows similar results obtained in another set of experiments, using cortisol acetate. It also shows the lack of inhibition by a weaker concentration of cortisol (10⁻⁸ M) and, for comparison, the inhibition effected by indomethacin (2 µg ml⁻¹). The inhibition produced by the latter was much greater than that effected by cortisol (10⁻⁶ M) and did not exhibit any latency.

To check further the dependence of the effect of cortisol on protein synthesis, experiments were carried out in which renal papillae were incubated in Krebs-Henseleit bicarbonate buffer (pH 7.4). Although PG biosynthesis proceeded unimpaired, as also described in previous publications^{11,12} cortisol (10⁻⁶ M) failed to alter PGE output during any of the incubation periods up to 3 h ($n=6$, results not shown). This observation contrasts with that on indomethacin, which also exerts its inhibition of PG synthetase in plain buffer incubations¹².

Table 1 Effect of cortisol and of inhibitors of RNA and protein synthesis on PGE output from rat renal papilla

Incubation period (min)	Control	Cortisol 10^{-6} M	Cortisol 10^{-6} M + actinomycin	Cortisol 10^{-6} M + cycloheximide	Cortisol 10^{-8} M	Indomethacin
0-30	438 ± 20	507 ± 81	486 ± 41	406 ± 36	469 ± 49	172 ± 23§
30-60	209 ± 12	171 ± 13	174 ± 15	119 ± 6‡§	205 ± 18	20 ± 2§
60-90	108 ± 10	68 ± 4*	101 ± 10‡	201 ± 17*‡	95 ± 8	10 ± 2§
90-120	99 ± 13	61 ± 5‡	110 ± 7‡	237 ± 20‡§	95 ± 13	7 ± 1§
120-150	103 ± 11	68 ± 6‡	128 ± 6‡	223 ± 17‡§	139 ± 19‡	4 ± 1§

PGE output expressed as ng per 100 mg wet tissue per 30 min, means ± s.e.m. of six experiments. DMEM containing the drugs specified was replaced every 30 min and PGE was determined by radioimmunoassay. Cortisol acetate was dissolved in ethanol and added to DMEM at a final alcohol concentration of 0.1%. Significant differences were determined using Student's *t*-test.

* $P < 0.005$ compared to control.

‡ $P < 0.025$ compared to control.

‡ $P < 0.01$ compared to cortisol 10^{-6} M.

§ $P < 0.001$ compared to control.

|| $P < 0.05$ compared to cortisol 10^{-6} M.

The output of PGs of the F type followed the same pattern as that of PGE and was similarly inhibited by cortisol (Table 2). As in the case of PGE, the inhibition of PGF production by cortisol (10^{-6} M) was prevented in the presence of either actinomycin D ($0.5 \mu\text{g ml}^{-1}$) or cycloheximide ($1 \mu\text{g ml}^{-1}$). The results shown in Table 2 also show that the ratio of PGE to PGF was not significantly altered by the presence of cortisol. This excludes a possible effect of cortisol on either endoperoxide reductase or isomerase¹³. It is also unlikely that the decreased outputs of PGE and PGF are secondary to effects on other PG pathways or on PG catabolism, as PGE₂ and PGF_{2 α} are the major PGs synthesised by the rat renal papilla, and their catabolism in this tissue is negligible^{14,15}.

To establish whether cortisol exerts its activity by inhibiting the synthesis of PGs in the renal papilla, or possibly their release, the PGE content in the tissue was determined. The papillary minces were homogenised and extracted into ice-cold diethyl ether immediately after 2 h of incubation. In the presence of cortisol (10^{-6} M), the content of PGE was 33 ± 5 ng per 100 mg wet tissue, compared with 77 ± 12 ng per mg in control experiments, indicating a 57% decrease in tissue content of PGE ($P < 0.005$). This suggests that cortisol inhibits the synthesis of PGs in the renal papilla, and agrees with data obtained in different preparations such as rheumatoid synovia⁶ or mouse fibrosarcoma cells⁹. Other authors, however, interpreted the decreased PG output from other tissues as evidence that corticosteroids inhibited the release, rather than the synthesis of PGs. Thus, inhibition by cortisol of PG release was assumed to have occurred in rabbit perfused mesenteric blood vessels¹⁶, perfused lungs of sensitised guinea pigs¹⁶, inflamed synovia^{7,17} and rabbit adipose tissue^{4,18}. However, some of these studies^{7,16-18} used cortisol concentrations (10 – $100 \mu\text{g}$

ml^{-1} , approximately 2.5×10^{-4} to 2.5×10^{-3} M) some 2 orders of magnitude higher than those known to produce physiological effects through specific steroid receptors, and may well involve less specific mechanisms, such as membrane stabilisation¹⁹. Thus, the corticosteroids at lower concentrations may inhibit PG biosynthesis, as indicated in the present and other reports^{6,9}, but at higher concentrations they may also interfere with membrane function and PG release^{7,16-18}.

Inhibitory or catabolic effects of the corticosteroids are not unusual and may involve the synthesis of inhibitory or toxic proteins²⁰. In the renal papilla, the mechanism of inhibition of PG synthesis by cortisol may involve either inhibition of synthetase or decreased precursor availability. The latter possibility is borne out by experiments in which inhibition by corticosteroids was prevented by the addition of precursor^{7,16,17}. Also, decreased precursor availability may be affected by increased utilisation of the precursor fatty acids, so that one need not assume the involvement of inhibitory proteins. Further work is required to ascertain the nature of the inhibition described here, and whether or not it is related to the anti-inflammatory or other effects of the corticosteroids.

Thus, the inhibition of PG synthesis by cortisol had a latency period, was prevented by inhibitors of RNA or protein synthesis, and could only be demonstrated in tissue culture medium, not in Krebs buffer. The inability of other investigators to observe effects of corticosteroids on PG biosynthesis in tissue homogenates¹, isolated microsomal preparations³ or buffer-perfused organs² becomes obvious if one accepts the fact that inhibition by low concentrations of corticosteroids requires protein synthesis. This also explains the effectiveness of corticosteroids in either blood-perfused organs⁴ or in tissue culture^{6,8,9}, where protein

Table 2 Effect of cortisol (10^{-6} M) on PGF output from rat renal papilla and on the ratio of PGE/PGF

Incubation period (min)	PGF output (ng per 100 mg per 30 min)			Control	PGE/PGF ratio Cortisol	Significance*
	Control	Cortisol	Significance*			
0-30	524 ± 57	591 ± 50	NS	1.08 ± 0.09	1.00 ± 0.06	NS
30-60	256 ± 37	192 ± 32	NS	0.85 ± 0.10	1.07 ± 0.12	NS
60-90	269 ± 55	96 ± 21	$P < 0.02$	0.64 ± 0.08	0.83 ± 0.15	NS
90-120	298 ± 53	130 ± 44	$P < 0.05$	0.65 ± 0.11	0.74 ± 0.16	NS

Data are means ± s.e.m. of six experiments. NS, not significant.

*Student's *t*-test.

synthesis can take place. The observation that cortisol was ineffective when added to isolated splenic strips, but could inhibit PG biosynthesis if the animals had been pretreated *in vivo*, is worth noting²¹.

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³¹P nuclear magnetic resonance studies of isolated rat liver cells

PHOSPHOROUS nuclear magnetic resonance (NMR) has recently been used in studies of intact cells, and many phosphate metabolites have been observed and identified. One valuable result of these studies has been the determination of intracellular pH (refs 1-3) using the fact that the position of the ³¹P NMR peak of inorganic phosphate (P_i) shifts as P_i is protonated. ³¹P NMR measurements of energised *Escherichia coli*³ have shown that a ΔpH is maintained across the membranes in agreement with previous measurements of ΔpH determined from the distribution of a weak acid, such as DMO, between the inner and outer volumes⁴. Although there is considerable evidence for the existence of ΔpH from studies of isolated mitochondria⁵⁻⁸, no direct measurements have been reported of a pH difference between cytosol and mitochondria in whole cells *in vivo*. However, there is indirect evidence for such a gradient from studies of the distribution of anions between the cytoplasmic and mitochondrial contents of disrupted rat liver cells⁹. In this preliminary report we show that ³¹P NMR can be used to detect phosphate metabolites in rat liver cells and to measure directly ΔpH between mitochondria and cytosol in the intact cell.

Figure 1a shows the ³¹P NMR spectrum of freshly isolated rat hepatocytes at 4 °C; the intracellular P_i resonance (peak C) consists of a main peak with a shoulder on the high field side.

The final wash used in preparing the liver cell pellet and the NMR resuspension medium were both phosphate-free. As confirmation that peak C consisted entirely of intracellular P_i, cells of a similar sample, with a similar NMR spectrum, were centrifuged for 6 min at 20g and the supernatant decanted. The ³¹P NMR spectrum of the supernatant, recorded in the same conditions as Fig. 1a, showed no detectable P_i.

The observation that peak C of Fig. 1a is not a simple singlet suggests that a ΔpH exists between the mitochondria and cytosol. To test this possibility, valinomycin was added to the rat liver cell suspension. Valinomycin has been shown to enhance ΔpH across the membrane in suspensions of isolated mitochondria⁶, where the uptake of K⁺ led to a reduction of the electrical potential across the mitochondrial membrane and to a net extrusion of protons. Thus, by analogy, a greater ΔpH between matrix and cytosol was anticipated in the rat liver cells. Figure 1b-f shows the evolution of the intracellular P_i resonance (peak C) into two discrete lines under the influence of valinomycin. In Fig. 2 the positions of the P_i NMR peaks are converted to pH; the time course of ΔpH in Fig. 2 is finer, as all the data are included. The effect of adding valinomycin is observed in the period between 45 and 127 min. Because lowering the external pH enhanced ΔpH in isolated mitochondria⁷, it was expected that lowering the external pH would increase the ΔpH in liver cells. At 136 min 10 μl of 0.5 M MES buffer at pH 3.3 was added to the cell suspension; and in Fig. 1g-h an even larger splitting of the P_i resonance can be observed, indicating a greater ΔpH. From the corresponding points in Fig. 2, it is clear that the cytosolic pH responded to the decrease in external pH, declining sharply from 7.10 to 6.91, whereas the mitochondrial pH remained relatively constant.

In contrast to the effect of valinomycin, a reduction in ΔpH between cytosol and mitochondria would be expected if the cells were de-energised by the action of the uncoupler FCCP or by prevention of respiration. Figure 1i shows the reduction of the P_i splitting caused by incubation of the cells with FCCP at 4 °C. After removing the oxygen supply and flushing with helium, we saw these two P_i lines collapse into one (Fig. 1j); this single peak became sharper in succeeding spectra. The corresponding pH changes are shown in Fig. 2. Although the effect of FCCP seems to be small in the present experimental conditions, studies at higher temperatures indicate that above 12 °C the same concentration of FCCP collapses ΔpH within the time required for an NMR spectrum (data not shown).

The two P_i resonances observed were assigned to mitochondria and cytosol on the basis of their responses to valinomycin, which increased ΔpH; to a reduced external pH, which mainly shifted the resonance assigned to cytosolic P_i; and to de-energisation, which collapsed ΔpH. As we have established that no peaks from external P_i were detectable, we have concluded that the two P_i peaks belong to the mitochondria and cytosol. That peak corresponding to the higher pH is assigned to the mitochondrial P_i and that corresponding to the lower pH to cytosolic P_i, as inferred from studies of isolated mitochondria⁵⁻⁸. Accurate concentrations cannot be derived from the present peak intensities because the radio frequency pulse repetition rate was not slower than the spin lattice relaxation rate.

Assignment of many of the remaining peaks in Fig. 1a to specific metabolites can be made on the basis of chemical shifts. Peak F at 5.4 p.p.m. arises from the resonance of the γ peak of ATP, whereas the β peak of ADP appears as a significant upfield shoulder. Peak G at 10.1 p.p.m. is composed of the α-ATP and α-ADP resonances. Peak H at 10.6 p.p.m. and peak I at 12.1 p.p.m. are in the P_iP'-diesterified pyrophosphate region. The position of the β peak of ATP (peak J) at 18.9 p.p.m. indicates that ATP in liver cells is bound to a divalent metal ion, probably Mg²⁺, and has been noted in Ehrlich ascites cells¹¹ and in muscles^{12,13}. Peak E at -0.49 p.p.m. is composed of endogenous and added glycerol-3-phosphorylcholine. The resonance at -1.02 p.p.m. (peak D) can be assigned to glycerol-3-

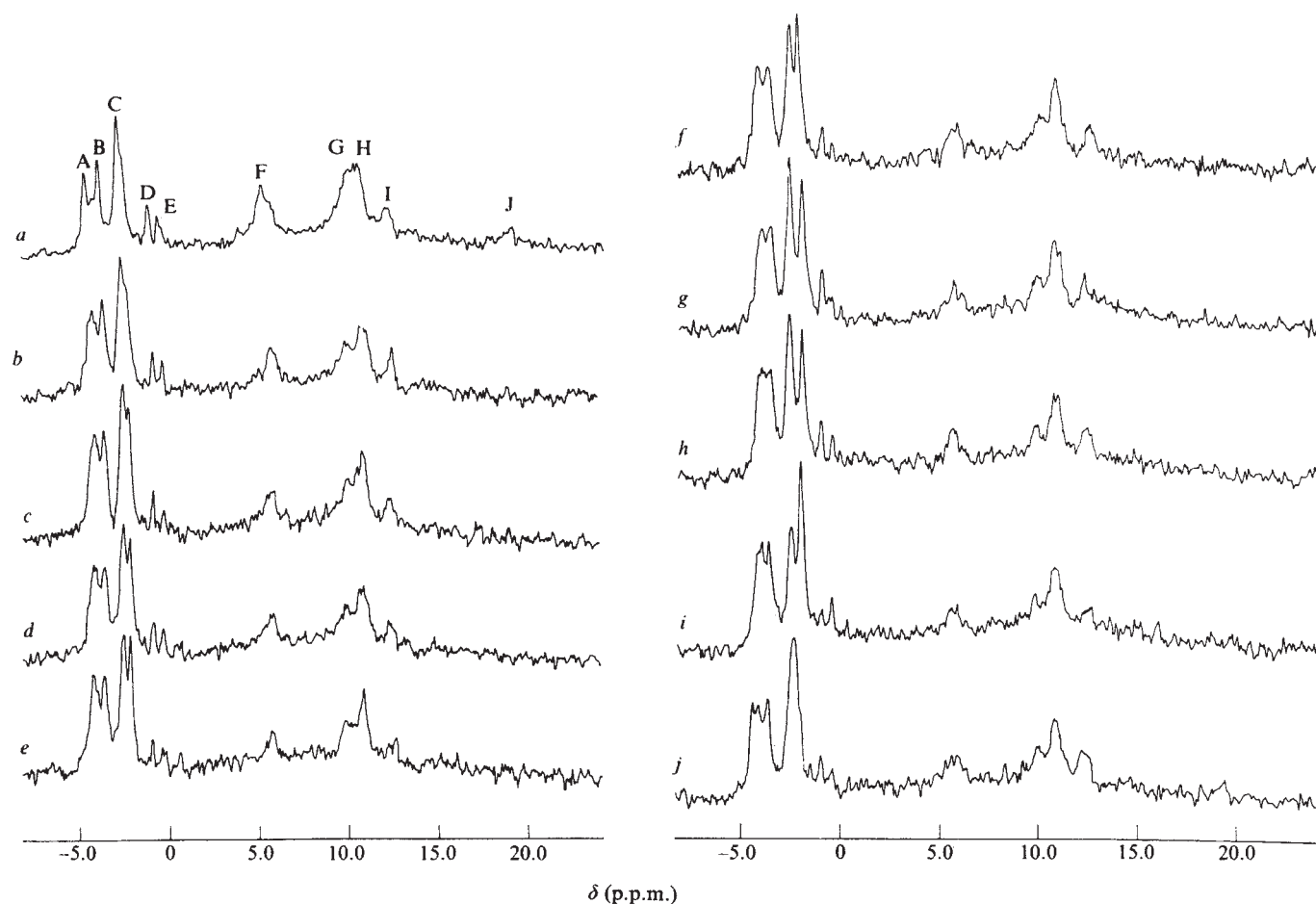


Fig. 1 NMR spectra at 145.7 MHz of ^{31}P nuclei in isolated rat liver cells. Each spectrum is the Fourier transform of the accumulation of 1,000 pulses of 60° free induction decays of 0.34s duration obtained with a Bruker HX 360 spectrometer. Male Sprague-Dawley rats fasted for 24 h were used for the isolation of the liver cells. The cells were prepared by the method of Berry and Friend¹⁴, with the modifications in the liver perfusion described by Tischler *et al.*⁹. Estimates of the viability of the cell preparation based on the number of cells that seemed to have normal morphology under light microscopy and the ability to exclude vital dyes indicated that initially 80–90% of the cells were viable and after the last spectrum was recorded 60–70% were viable. The cells were resuspended in three times their volume of a phosphate-free Krebs bicarbonate medium of pH 7.3 (120 mM NaCl, 24 mM NaHCO_3 , 4.8 mM KCl, 1.2 mM MgSO_4 , 1.3 mM CaCl_2 , 5.5 mM D-glucose) to a final volume of 2 ml and equilibrated with a gas mixture of 95% O_2 /5% CO_2 at 4 °C. All chemical shifts are given with respect to orthophosphoric acid at 0 p.p.m. 1 mM glycerol-3-phosphorylcholine, peak E, was added as an internal marker at -0.49 p.p.m. Measurement of the first spectrum was started at time zero. *a*, An early spectrum of the isolated rat liver cells at time 27 min before any additions. Spectra *b–f* were taken after bringing the sample to 10^{-5} M in valinomycin. Spectra *b, c, d, e* and *f* were started at 64, 73, 91, 100 and 127 min, respectively. Spectra recorded immediately after the addition of 10 μl of 0.5-M MES buffer at pH 3.3 to the liver cell suspension are shown in *g* and *h*, which correspond to time 145 and 154 min, respectively. Cell spectra taken after bringing the sample to 10^{-5} M in FCCP are shown in *i* and *j*, which were recorded at 200 and 236 min, respectively. The O_2/CO_2 supply was replaced by helium before spectrum *j* was measured. In an NMR spectrometer using an electromagnet, the external magnetic field, H_0 , is perpendicular to the long axis of the cylindrical NMR sample tube and the coaxial capillary contains an external standard such as H_3PO_4 . In super-conducting magnets H_0 is parallel to this axis. Consequently there are differences in the corrections coming from the bulk magnetisation on comparing chemical shifts obtained with an external coaxial standard when different H_0 sample geometries are used. In typical aqueous solutions the former chemical shifts are about 0.73 p.p.m. higher than the latter, which are quoted here (for discussion see ref. 10).

phosphorylethanolamine. Peak B at -3.7 p.p.m. is at the position expected for phosphorylcholine¹¹. The peak A region encompasses several sugar phosphates, but any assignments of these peaks must await titration of cell extracts. In addition to the effects observed above, we would expect valinomycin to cause the ATP level to decrease, because valinomycin partially uncouples phosphorylation and probably also stimulates $\text{Na}^+\text{-K}^+$ ATPase by inducing a K^+ leak from the cytosol to outside medium and mitochondria. In Fig. 1*b–j*, the disappearance of ATP after the addition of valinomycin can be observed. The β -ATP resonance (peak J) is absent and peak F now consists mainly of β -ADP, which appears further upfield at

5.6 p.p.m.; peak G is now mainly the α -ADP resonance which appears slightly downfield at 9.7 p.p.m. and consequently is better separated from peak H than was the α -ATP peak.

In conclusion, high resolution ^{31}P NMR has been used to observe and assign resonances of major phosphate metabolites in rat liver cells and to determine both mitochondrial and cytosolic pH values in the respiring cell. These observations indicate the potential of this method for studying the distribution of metabolites between mitochondria and the cytosol in whole liver cells.

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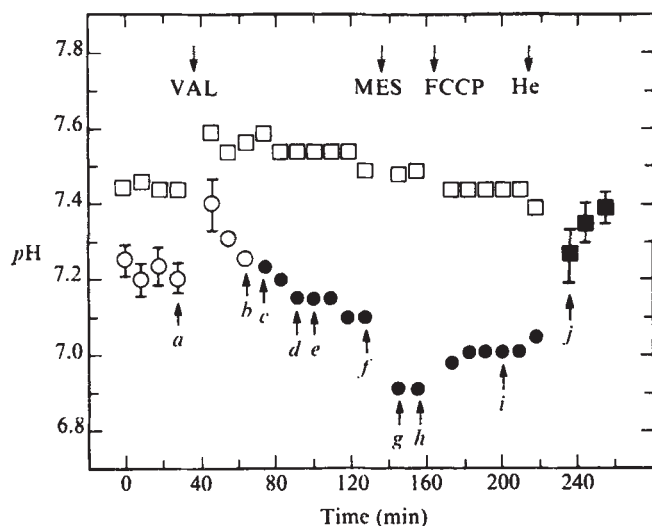


Fig. 2 Changes in cytosolic and mitochondrial pH in isolated liver cells derived from NMR spectra shown in part in Fig. 1. All experimental conditions are as given in Fig. 1. The NMR resonance positions of P_i (peak C in Fig. 1) were converted to the pH scale using as a calibration the titration of P_i , measured at 4 °C, in a solution containing 150 mM K^+ , 30 mM HCO_3^- , 10 mM P_i , 110 mM Cl^- , 5 mM Mg^{2+} , 16 mM glycero-3-phosphorylcholine, and 3 mM ATP, designed to approximate the salt conditions in the cytoplasm. The downfield, high pH, peak is identified as mitochondrial P_i (●); the upfield, lower pH, component of peak C is assigned to the cytosolic P_i (○). Before peak C is clearly split into two discrete lines by the presence of valinomycin, an upfield shoulder can be observed on peak C (see Fig. 1a). All peak positions in such cases were determined by convolution difference spectroscopy¹⁵ and the initial cytosolic pH estimated from the result (○). Arrows denote the addition of valinomycin, MES buffer, FCCP and the substitution of helium for the O_2/CO_2 supply at the indicated times. The coalescence of the cytosolic and mitochondrial P_i resonances after the cells were de-energised by the addition of FCCP and the use of helium is also indicated (■). a-j, Corresponding spectra of Fig. 1. Error bars, which were absent fall within the symbol, represent our best estimates of reliability.

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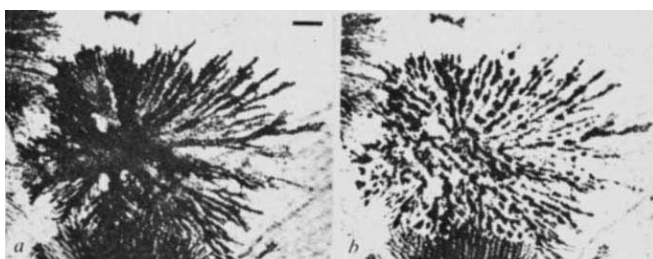
A microtubule-independent component may be involved in granule transport in pigment cells

COLOUR change in lower vertebrates is based on the movement of thousands of pigment granules within integumental chromatophores. In some types of pigment cells, particularly those of teleost fish, pigment aggregation in the cell centre and redistribution into the cell processes may take place within fractions of a minute^{1,2}. These cells may therefore be a suitable model system for the study of intracellular vesicle transport. There is considerable information about the structure of chromatophores, in particular their prominent microtubule system²⁻⁵, but the mechanism of pigment movements remains obscure. Pigment granule transport is thought to be microtubule-dependent⁶ and microtubules definitely play a part in this process^{1,2,6}. However, it remains to be determined whether these organelles also directly provide the driving force for the movements. The crucial question is: what happens when the microtubule frame is withdrawn experimentally? We therefore tested the ability of chromatophores to move pigment granules in conditions where microtubules were absent, and further investigated the nature of the physical relationship between microtubules and pigment granules. We now report the presence of a second component involved in the transport process.

Melanophores of *Pterophyllum scalare* and *Gymnocorymbus ternetzi* were used either *in situ* (within an isolated scale) or *in vitro* (after isolation from surrounding tissue). The method of isolation and short-term culture of melanophores spread on coverslips is described elsewhere⁷. Adrenaline (10^{-4} M) and atropine (10^{-4} M), respectively, were used to elicit the centripetal and centrifugal movements of pigment granules.

Treatment of melanophores with temperatures around 0 °C led to a complete loss of microtubules which further did not reappear within the first 2 min of release from cold treatment, as shown by indirect immunofluorescence microscopy using tubulin antibodies and electron microscopy (data not shown). If, concomitantly with rewarming the cells, adrenaline was applied, numerous small granule clusters formed throughout the cell within the first minute (Fig. 1). During the following minutes these microaggregates gradually fused and ultimately formed a central pigment mass. While these latter steps took place in the presence of microtubules, the initial formation of microaggregates seemed to be a microtubule-independent process.

Fig. 1 Scale melanophore of *Gymnocorymbus*. a, After 30 min of cold treatment. b, Same cell after 1 min recovery and administration of adrenaline. Scale bar, 10 μ m.



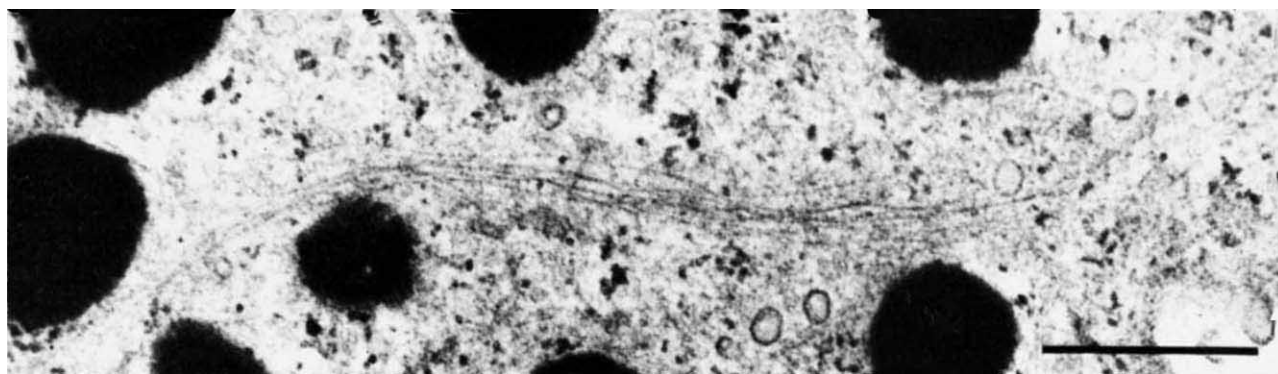


Fig. 2 Isolated melanophore of *Pterophyllum* fixed and sectioned for conventional electron microscopy. Fixation with 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 30 min was followed by 2% osmium tetroxide made up with potassium bichromate and sodium chloride⁹. The cells were dehydrated in ethanol and embedded in an Epon-Araldite mixture. Sections were stained with lead citrate and viewed in a Hitachi H 500 electron microscope. The section of the cell shown here is exactly parallel to the substrate surface. Cold-plus-colchicine treatment was followed by 30 min recovery from cold treatment. Filaments were intermediate in size (7–10 nm). No microtubules are present. Scale bar, 0.5 μ m.

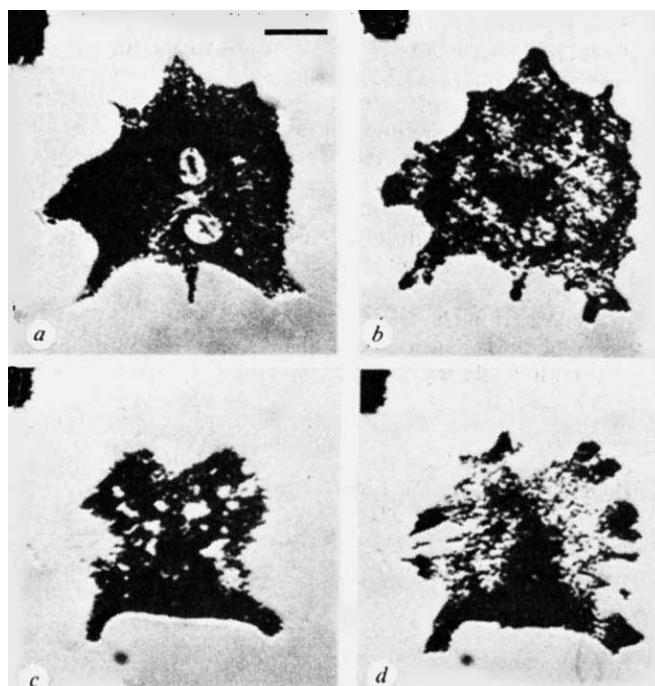
In an attempt to remove the microtubule frame permanently we combined a 30-min cold shock with colchicine treatment. Unlike colchicine treatment alone, where microtubules are still present even after several hours^{8,9} the cold-plus-colchicine combination depolymerises all microtubules, guarantees the binding of colchicine to tubulin subunits, and prevents the reformation of microtubules. In experiments with isolated cells a colchicine concentration of 5×10^{-5} M was used. On rewarming the cells, pigment granules remained more or less evenly distributed throughout the cell and showed slow movements similar to the 'shuttle' movements of untreated cells, except that there was no preferential direction. Cells fixed after a 30 min rewarming period were completely devoid of microtubules. Instead, a much higher than normal number of intermediate-sized (7–10 nm) filaments was observed (Fig. 2). They coursed through the cytoplasm in small intermingling bundles in a prevailing radial direction. If adrenaline was added shortly after rewarming, an aggregation-like reaction was observed within about 10 min which could be readily reversed with atropine (Fig. 3).

The experiments seem to rule out a mechanism of pigment movement in which the driving force is provided solely by microtubules. As in the maintenance of the aggregated state¹⁰, another component seems to be involved that clearly depends largely on the presence of microtubules to produce ordered movements. We therefore attempted to elucidate the morphological and chemical nature of this 'second component'. Conventional electron microscopy shows, apart from microtubules, a fibrous and diffuse cytoplasmic matrix and no clear evidence of microfilaments³. To gain an insight into the three-dimensional organisation of this matrix, isolated melanophores were prepared for high voltage electron microscopy using the techniques developed by Buckley¹¹ and Woloszewick and Porter¹² (see legend to Fig. 4). Stereo pairs (Fig. 4) showed the matrix component to be arranged in a system of fine fibrillar strands (microtrabeculae) connecting microtubules, melanosomes and the plasma membrane. This trabecular system, which is similar to that observed in erythrophores of *Holocentrus ascensionis*¹³, changes dynamically in association with granule displacements, and its interaction with microtubules as guiding elements may be responsible for the directional movements. In the cold-plus-colchicine experiments, where microtubules were absent, granule movements were impaired (Fig. 3), but some sort of guidance was possibly provided by the intermediate-sized filaments which had hardly any active part in the transport process. Their increased numbers after colchicine treatment are consistent

with observations in other cell types where a proliferation of these filaments is caused by antimicrotubular agents¹⁴.

The chemical nature of the second component is at present poorly understood. To identify the possible presence of an actomyosin system, a likely candidate for a component engaged in motility, cells with and without intact microtubules have been treated with cytochalasin B, a drug interfering with some actin-related functions¹⁵. Cells with intact microtubules still aggregated and dispersed their pigment granules in response to appropriate stimuli at rates indistinguishable from controls after up to 90 min of treatment with $5\text{--}20 \mu\text{g ml}^{-1}$ cytochalasin B. In comparison to normal cells, in cytochalasin B-treated cells there was some clumping of pigment granules in the cell processes which, however, did not impair centripetal granule transport. This lack of inhibitory action of cytochalasin B in fish melanophores, also noted by Ohta¹⁶, is in marked contrast with

Fig. 3 Isolated *Pterophyllum* melanophore after 6 h of culture. *a*, Normal cell at room temperature. *b*, After 30 min in 0°C in the presence of 5×10^{-5} M colchicine. *c*, After 10 min of recovery from cold treatment in the presence of adrenaline, followed by *d*, 10 min atropine. Scale bar, 10 μ m.



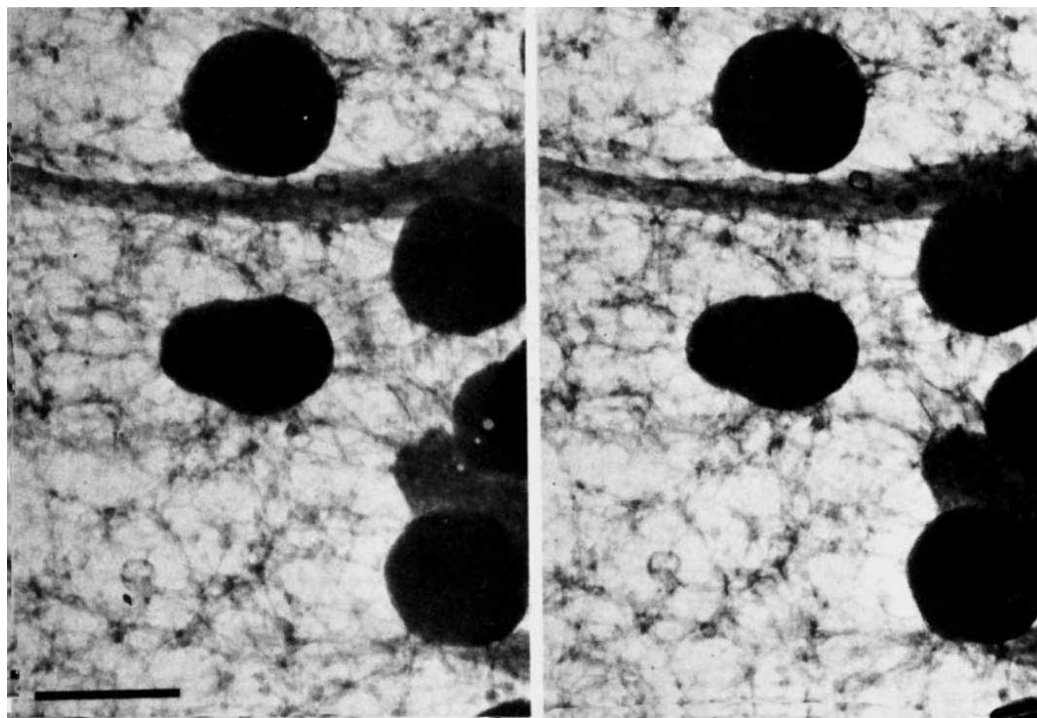


Fig. 4 High-voltage electron micrograph of a region immediately adjacent to the pigment mass of a *Pterophyllum* melanophore fixed shortly after the onset of dispersion. Isolated cells were allowed to settle on Formvar and carbon-coated gold grids. After 2–8 h of culture⁷, cells in the desired state of pigment dispersion were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 20–30 min and 2% osmium tetroxide in cacodylate buffer for 5–8 min. Following rapid dehydration in acetone, cells were critical-point dried using Sorvall equipment. A carbon coat was added to further stabilise the cells, which were then viewed in a Jeol 1,000-kV electron microscope. In the cell shown here, the pigment-free cell periphery is to the left. This stereo pair shows the extensive microtrabecular system associated with pigment granules. A stereoscopic binocular viewer should be used to visualise the stereo images. Without this aid, stereoscopic viewing may be achieved by eye convergence. Tilt angle 4°. Scale bar, 0.5 μ m.

similar experiments with amphibian melanophores^{17,18} and sea urchin chromatophores¹⁹. On the other hand, cytochalasin B at 5 and 10 μ g ml⁻¹ completely abolished the aggregation response (see Fig. 3) of cold-plus-colchicine-treated cells. The significance of this inhibition is not clear; possibly the drug acts on a component that becomes cytochalasin B-sensitive only after removal of microtubules. This view is further supported by experiments where cytochalasin B-treated melanophores (10 μ g ml⁻¹) are cooled for 30 min to 0 °C. After this transient removal of microtubules, the time required for adrenaline-stimulated cells to reach the aggregated state is prolonged by a factor of 2 when compared with cells without cytochalasin B-addition (data not shown).

In further experiments, glycerinated melanophores were treated with heavy meromyosin (HMM) or HMM-subfragment 1 to test for the possible existence of actin filaments. However, experiments carried out in a variety of conditions have not so far revealed the presence of actin.

Thus it seems that an as yet poorly understood component is engaged in granule transport within teleost melanophores. Its interaction with microtubules as essential guiding elements may bring about rapid, directional movements.

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Biochemical evidence that leghaemoglobin genes are present in the soybean but not in *Rhizobium* genome

LEGAEMOGLOBIN (Lb), a haem protein with structural and functional similarities to animal myoglobin¹, is found uniquely in legume root nodules, the sites of nitrogen fixation which form during symbiotic association between legumes and soil bacteria of the genus *Rhizobium*. Although in certain conditions free-living *Rhizobium* can express nitrogenase activity in the absence of Lb (see, for example, ref. 2), the presence of the protein seems to be required for the development of nitrogenase activity by *Rhizobium* bacteroids within legume root nodule cells. Lb probably functions by transporting oxygen to *Rhizobium* bacteroids, at the same time maintaining a low partial pressure of oxygen to protect nitrogenase from oxygen-inactivation.

Genetic evidence indicates that the type of Lb found in root nodules is a characteristic of the plant host rather than the infecting *Rhizobium*^{3,4}. However, as it has not been possible to detect Lb in legume tissues in the absence of *Rhizobium*, biochemical proof of the location of the Lb genes has been lacking. We report here that DNA complementary to Lb messenger RNA (Lb cDNA) hybridises with soybean but not with *Rhizobium* DNA.

Hybridisation between Lb mRNA and Lb cDNA prepared as described in the legend to Fig. 1 was measured by

S-1 nuclease resistance, hydroxyapatite (HAP) chromatography and equilibrium centrifugation in CsCl as described by Harrison *et al.*⁷. Although only about 40% of the cDNA hybridised to Lb mRNA, in parallel experiments 80% of mouse globin cDNA hybridised to mouse globin mRNA. After reacting Lb mRNA with cDNA, hybrid molecules were separated by HAP chromatography and hybridisable cDNA was isolated by centrifugation in CsCl after alkaline hydrolysis of the hybrids. The degree of hybridisation of this fraction of cDNA to Lb mRNA was not significantly different from that obtained with the original cDNA. Because, as we will show, the Lb cDNA hybridises to a much greater extent (about 75%) with soybean DNA, it is likely that the degree of Lb mRNA-Lb cDNA hybridisation is a property of the mRNA rather than the cDNA. This problem has been encountered by others⁸ and it possibly results from mRNA secondary structure which could interfere with hybrid formation⁹.

Figure 1 shows that significant annealing of Lb cDNA occurs with soybean DNA (Fig. 1a) but not with *Rhizobium* (Fig. 1b) or wheat (Fig. 1c) DNA. Although 10–15% of the cDNA annealed with *Rhizobium* DNA at high C_0t values, similar values were obtained when Lb cDNA was reacted with DNA from wheat and *Escherichia coli* (data not shown). This can be accounted for by some self-annealing of the cDNA (dashed curve in Fig. 1a) and the possible non-specific binding of cDNA to either DNA or HAP, as described elsewhere⁷. The wheat DNA-Lb cDNA control also shows that there is no appreciable nonspecific sticking of cDNA to plant DNA. Because it is likely that the cDNA mostly contains faithful, partial copies of Lb mRNA, at

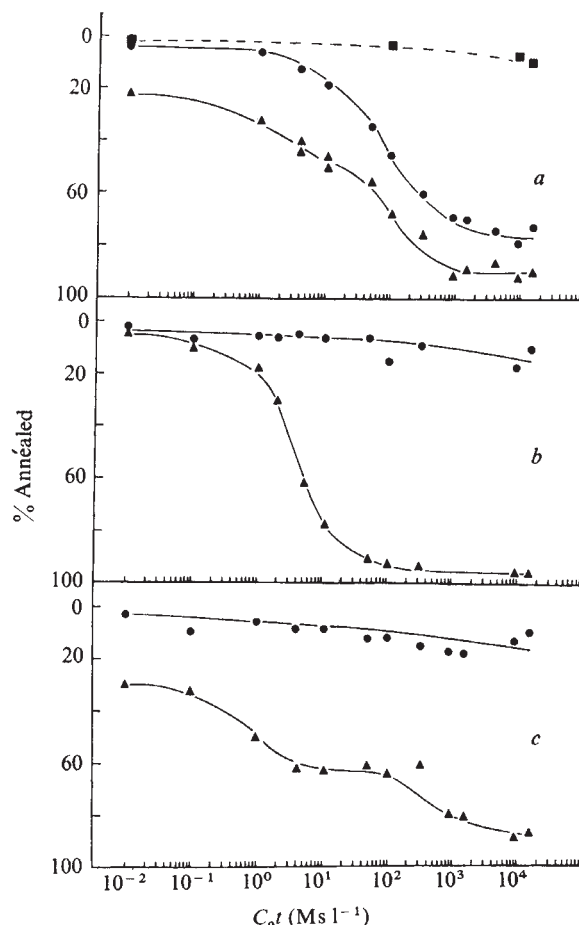
least 70% pure⁸, these results show that Lb genes are present in soybean but not *Rhizobium* DNA. The reason for almost 25% of the tracer cDNA not annealing with soybean DNA is obscure. In general, in DNA excess experiments, tracer nucleic acid does not hybridise as completely as driver DNA, even when conditions of sufficient DNA excess and equal fragment lengths are taken into consideration¹⁴.

The curve in Fig. 1 showing the kinetics of soybean DNA reassociation can be resolved into three components; a rapidly annealing one having a C_0t value of less than 10^{-2} , representing 20% of the genome and containing highly repetitive DNA sequences; an intermediate one representing about 35% of the genome and probably containing DNA sequences with a range of repetition frequencies; and a more slowly annealing component representing about 45% of the genome which probably contains the unique sequences of soybean DNA. This complexity is similar to the renaturation kinetics of wheat DNA shown in Fig. 1c, reported elsewhere^{13,15–17}.

The curve in Fig. 1 describing the kinetics of hybridisation of Lb cDNA to soybean DNA can be resolved into a minimum of two components, each of which follows second order kinetics. The more rapidly hybridising component (or the broadness of the curve beyond two logs of C_0t) may have resulted from the size of our DNA fragments, for Harrison *et al.*⁷ have shown that mouse DNA fragments of less than 120 nucleotides long must be used before globin cDNA anneals totally with a $C_0t_{1/2}$ expected for nonrepetitive sequences. The more slowly hybridising component, representing about 70% of the hybridisable cDNA, has a

Fig. 1 Annealing of trace amounts of radioactive Lb cDNA to nonradioactive DNA isolated from a, soybean, b, *Rhizobium* and c, wheat; $\blacktriangle$, renaturation of total, nonradioactive DNA; $\bullet$, annealing of radioactive Lb cDNA to excess nonradioactive DNA; $\blacksquare$, Lb cDNA self-annealing. Soybean Lb mRNA, purified as described previously⁸, was used to synthesise a complementary DNA in a 250- μ l reaction mixture containing 4 μ g of Lb mRNA, 12.5 μ g of oligo (dT)^{12–18} (Collaborative Research), 25 μ g of actinomycin D, 23 units of AMV RNA-dependent DNA polymerase (Office of Program Resources and Logistics), 10 mM dithiothreitol, 6 mM magnesium acetate, 50 mM KCl, 50 mM Tris-HCl, pH 8.2, and 40 μ M each of dATP, dTTP, dGTP and d^{(3)H}-CTP (24.1 Ci mmol⁻¹). After incubation at 40 °C for 60 min the reaction mixture was fractionated on Sephadex G-50. The average size of the cDNA was approximately 400 nucleotides long, as determined by centrifugation in alkaline sucrose gradients⁸. DNA was purified from *Rhizobium japonicum* strain 61A76, grown as previously^{10,11}. Following the ribonuclease treatment of his procedure the preparation was digested with 500 μ g ml⁻¹ of proteinase K (Merck) for 2 h at 37 °C. It was then extracted with chloroform:isoamyl alcohol (24:1) until there was no precipitate at the interface and isopropanol precipitation of the DNA was omitted. To purify soybean (*Glycine max* var. Kanrich) DNA, dry embryos were ground to a powder and nuclei were isolated by the method of Stern¹². DNA was extracted from the nuclei by the same procedure used with *Rhizobium*, but in addition to ribonuclease the preparation was also treated with α -amylase¹³ before proteinase K digestion and banded in CsCl equilibrium density gradients⁷. Wheat DNA was prepared from ground non-toasted wheat germ (General Mills) by the same method used for *Rhizobium*. The DNA preparations were sonicated and sized in alkaline sucrose gradients⁷. Fractions corresponding to the size of the Lb cDNA (approximately 400 nucleotides long) were pooled, neutralised and dialysed against distilled H₂O. Nonradioactive DNA was dissolved at a concentration of either 5 mg ml⁻¹ (for $C_0t > 1.0$) or 0.05 mg ml⁻¹ (for $C_0t \leq 1.0$) in renaturation buffer⁷ which contained Lb cDNA (7200 c.p.m. per ng) at a concentration of 28.3 ng ml⁻¹. To measure cDNA self-annealing, nonradioactive DNA was omitted. 20- μ l aliquots were sealed in glass capillaries and heated in a boiling water bath for 5 min to ensure complete denaturation, and then incubated at 60 °C. At various times 20- μ l aliquots were removed, diluted with 0.02 M sodium phosphate, pH 6.8, containing 0.1 M NaCl and stored at -20 °C until they were analysed. Analyses were done with hydroxyapatite (Biogel HTP from Bio-Rad) at 60 °C by applying the samples in 0.02 M sodium phosphate, pH 6.8, and 0.1 M NaCl, and eluting single and double stranded material with 0.14 M and 0.4 M sodium phosphate, pH 6.8, respectively⁷. The fractions were adjusted to pH 13 with 25% NaOH, and the amount of DNA in each was determined by their absorbance at 260 nm.

The amount of Lb cDNA was determined by measuring the radioactivity of each fraction after dispersal in Instagel (Packard).



$C_{0t_{1/2}}$ identical to that for the slowly annealing component of the soybean DNA reassociation curve (1.4×10^3). For reasons which we do not understand the soybean DNA in our experiments renatured about 10-fold faster than has been reported by Hepburn *et al.*¹⁶ and Goldberg¹⁷. Nevertheless, by comparing the kinetics of renaturation of mouse and soybean DNA in identical conditions and taking into account the differences in haploid genome size of mouse and soybean, the $C_{0t_{1/2}}$ of 1.4×10^3 for the soybean DNA-Lb cDNA hybridisation reaction is well within the range expected if the Lb genes are repeated with a similar frequency to mouse globin genes⁷, suggesting that the genes specifying leghaemoglobin are probably within region(s) of unique sequence soybean DNA.

In addition to providing biochemical evidence for the location of leghaemoglobin genes in the plant host, Lb cDNA should prove valuable for analysing leghaemoglobin gene activity during the development of nitrogen-fixing root nodules.

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Preparation-sensitive conformations in ribosomal RNAs

A METHOD has already been described for isolating RNA from the small subunit of the *E. coli* ribosome which had altered conformation by the following criteria: additional protein binding sites^{1,2}, sensitivity to T_1 RNase³ and mobility on polyacrylamide-Agarose electrophoresis³. We also showed that it was possible to detect the conformational difference by electron microscopy⁴. Even though the details of the structure were either below the resolution of the electron microscope, or not sufficiently homogeneous to describe in detail, length measurements of the molecules gave a statistically significant description of the structure. Previously, the only significant ribosomal RNA structure that could be resolved in the electron microscope was the characteristic secondary structure of RNA of the large subunit of eukaryotic ribosomes, or the precursor to eukaryote rRNA⁵. We wished to find out whether, generally, in rRNAs other than the 16S of *E. coli*, one could detect additional structure in the electron microscope, the details of which may be beyond the resolving power, and whether there are preparation-sensitive conformations in ribosomal RNA. The two preparation procedures used correspond to those described¹, acetic

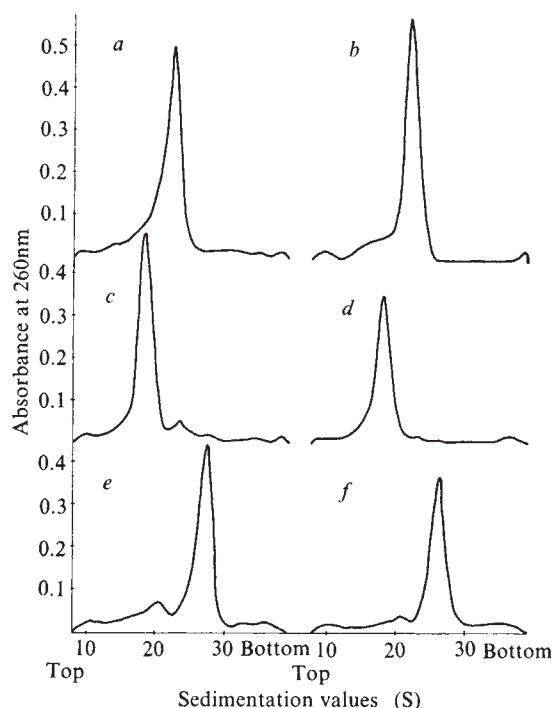


Fig. 1 Analytical sucrose gradients of the rRNA preparations. The RNA preparations were from *E. coli* 50S subunits (a, b) prepared according to Gordon and Ramjouw⁶ or from chick liver 40S (c, d) and 60S (e, f) subunits prepared according to Ramjouw and Gordon⁷. The rRNA^a (a, c, e) and rRNA^p (b, d, f) preparations were according to Hochkeppel *et al.*¹. Gradients (5.1%-28.6% w/v sucrose, isokinetic buffer: 0.2% sodium dodecyl sulphate, 100 mM LiCl, 10 mM Na₂EDTA, 10 mM Tris-HCl, pH 7.4) were run for 90 min at 60,000 r.p.m. in the Beckman SW60 rotor at 20 °C. Each gradient was loaded with approximately 0.2 A_{260} per cm³ of the RNA preparation.

acid-urea extraction (rRNA^a) and the conventional phenol extraction (rRNA^p). We show here that there are in fact previously unrecognised structural elements in a variety of ribosomal RNAs.

We previously⁴ defined a structure parameter, the 'extension factor', which described the amount of structure in a molecule as the ratio of the formaldehyde-denatured length to the non-denatured length. We have now extended this analysis to other kinds of ribosomal RNA, namely the 23S rRNA of the large subunit of the *E. coli* ribosome, and the 18S and 28S rRNAs of the chick liver small and large ribosomal subunits. First, we established the integrity and purity of these RNA preparations by sucrose gradient analysis (Fig. 1). All the RNA preparations sedimented according to their nominal S values, and, as had also been shown for the 16S RNA^{2,4}, the sedimentation was preparation-insensitive. In addition, we heated the RNA in conditions⁸ known to reveal hidden breaks (0.01 M Tris-HCl, pH 7.4, 0.1 M NaCl, 0.01 M EDTA, 5 min, 60 °C) and found no effect on the sedimentation profiles of any of the RNA preparations (data not shown). We also analysed RNA for possible residual ribosomal proteins by running on heavily loaded polyacrylamide gels, as described by Thomas *et al.*⁹, and stained for protein.

For *E. coli* 23S and chick liver 18SRNAs, there was no detectable stain, giving a confidence limit of $\ll 0.1\%$ of the original ribosomal protein. The 28S RNA contained material which was on the limit of detection, and by densitometry, was found to be approximately 1.5% of the original ribosomal protein for the 28S RNA^a and approximately 2.5% of the original ribosomal protein for the 28S RNA^p. The stain was dispersed diffusely, and therefore did not correspond to an individual protein. From this we can be confident that the structure in the electron microscope is not subject to artefacts of fragmentation or protein contamination.

Therefore, we made a statistical analysis of the length

Table 1 Electron microscopy of various rRNAs

	-HCHO	Mean length (μm) +HCHO	Literature 0.68 (ref. 4) 0.70 (ref. 4), 0.57 (ref. 10)	Extension factor 8.3 (ref. 4) 4.6 (ref. 4)
16S RNA ^a				
16S RNA ^p				
23S RNA ^a	0.30 $\pm$ 0.08 (154)	1.16 $\pm$ 0.19 (70)		3.9
23S RNA ^p	0.58 $\pm$ 0.15 (127)	1.20 $\pm$ 0.18 (70)	1.00 (ref. 10)	2.1
18S RNA ^a	0.29 $\pm$ 0.07 (197)	0.98 $\pm$ 0.05 (70)		3.3
18S RNA ^p	0.49 $\pm$ 0.07 (129)	0.97 $\pm$ 0.05 (66)	0.60 (ref. 11)	2.0
28S RNA ^a	0.76 $\pm$ 0.07 (101)	1.20 $\pm$ 0.12 (60)		1.6
28S RNA ^p	0.87 $\pm$ 0.10 (104)	1.18 $\pm$ 0.15 (64)	1.43 (ref. 11)	1.4
TMV RNA ^a	1.93 $\pm$ 0.11 (50)	ND		
TMV RNA ^p	1.96 $\pm$ 0.09 (51)	ND	2.12 (ref. 12)	

Length are means $\pm$ s. d. for the number of individual measurements shown in parentheses. Extension factor is (length of formaldehyde treated)/(length of untreated). Preparations were as described for Fig. 1. For comparison, single strand chain lengths of the same RNA species from the literature are given, as well as our own data for the *E. coli* 16S RNA^a. ND, not determined.

distributions of the RNA preparations. A typical set of size distribution curves is given in Fig. 2 for the *E. coli* 23S RNA, and shows that the 23S RNA^a was significantly smaller than the 23S RNA^p (Fig. 2a and b, respectively), and that they were both smaller than the corresponding denatured forms (Fig. 2c and d). The complete summary of the statistical analysis of all the RNA preparations is given in Table 1, including previously published data for comparison. It can be seen that the dimensions of both

the *E. coli* 23S rRNA and the chick liver 18S rRNA were preparation-sensitive (compare each RNA^a and RNA^p, -HCHO). The 28S rRNA was preparation-insensitive; in this case, the well known secondary structure features (which have also been described for chicken 28S rRNA¹¹) were present following both preparative procedures, and no additional feature was revealed by the acetic acid procedure. After formaldehyde treatment, all RNA sizes became preparation-insensitive (compare each RNA^a and RNA^p, +HCHO). In addition, all RNAs had significant structure that was sensitive to formaldehyde (compare -HCHO and +HCHO, and extension factors all > 1). The mean lengths of the corresponding pairs of formaldehyde-treated RNA preparations (compare RNA^a, RNA^p, +HCHO) agreed with each other within very close limits, giving an independent corroboration of the precision of the procedure.

The tobacco mosaic virus (TMV) RNA^a and TMV RNA^p also yielded mean lengths which agreed with each other within very close limits, showing that for this RNA there is probably no preparation-sensitive conformer possible. Thus, in general, preparation-sensitive conformers may be characteristic of ribosomal RNAs, and not a feature of viral RNAs. This remains to be verified.

All of our length measurements of formaldehyde-treated RNAs were systematically higher than the published values for the single-stranded chain lengths, except in the case of 28S rRNA (see Table 1). This is consistent with the observation of Wellauer and David⁵ that the structure of 28S rRNA is relatively resistant to formaldehyde denaturation. This limits the usefulness of formaldehyde in the definition of the extension factor. Since completing this work, we have become aware of the detailed description of the application of glyoxal as an irreversible RNA denaturing agent¹³. This will then be preferable to formaldehyde in the future.

Regardless of the absolute values for the polynucleotide chain lengths, our results leave no doubt that interesting preparation-sensitive structures exist in a variety of ribosomal RNAs. These may well be metastable conformations which preserve the memory of RNA-protein interactions, as H.-K.H. and Craven¹⁴ have shown to be the case for 16S rRNA from *E. coli*.

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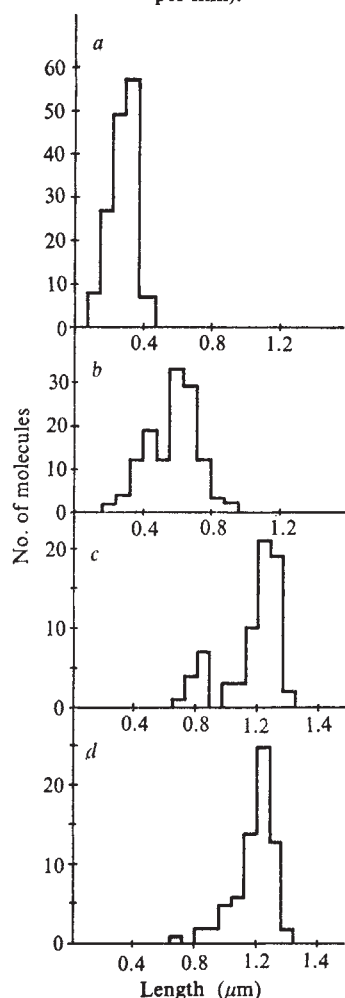


Fig. 2 Histograms of the length distributions of 23S rRNA preparations. a, 23S RNA^a; b, 23S RNA^p; standard spreading as described by Wellauer and David⁵. c, 23S RNA^a; d, 23S RNA^p; first denatured by heat treatment in 3% formaldehyde for 20 min at 63 °C (ref. 5). The spreading of the molecules in this work was such that the simplified 'end-to-end' procedure previously described⁴ was not suitable. The precise contour length of each molecule on the electron micrographs was therefore measured with a projector and electronic digitiser (Numonics). The RNA preparations were as in the legend to Fig. 1. Pictures were taken in a Siemens Elmiskop IA. The magnification of the instrument was calibrated with a carbon grating replica (Ladd, 2,160 lines per mm).

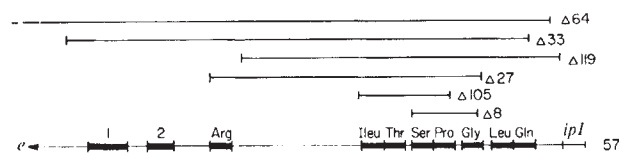
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***In vitro* transcription and isolation of a polycistronic RNA product of the T4 tRNA operon**

ALTHOUGH the expression of bacteriophage T4 genome has been extensively studied¹, no direct relationship has been established between specific genes and their immediate transcripts. *Escherichia coli* RNA polymerase transcribes *in vitro* a T4 DNA region of about 50 genes which tend to cluster according to function² and are expressed early after bacteriophage infection. One of these clusters is the tRNA region coding for eight tRNA species and two stable RNA species of unknown function which appear after bacteriophage infection^{3,4}. Deletion mutants lacking different sequences in this region have been isolated and well characterised⁵ (Fig. 1). T4-specific tRNAs can be produced *in vitro* by transcribing T4 DNA with *E. coli* RNA polymerase and processing the unfractionated product with *E. coli* extracts⁶. Although the temporal order of appearance of T4 tRNA species in the *in vitro* transcription-processing system suggested that the tRNA genes may be organised as a single transcription unit⁷, the formation of a long polycistronic precursor was not demonstrated. Here we describe the fractionation of RNA transcripts produced on T4 DNA by *E. coli* RNA polymerase into several high molecular weight RNA species. Using as templates DNA from T4 mutants containing deletions in the tRNA gene region we identified a polycistronic precursor of T4 tRNAs among the primary transcription products. This precursor was isolated and processed into mature size tRNA molecules.

Wild-type T4 DNA and DNA from six different tRNA deletion mutants were transcribed *in vitro* by *E. coli* RNA

Fig. 1 T4 tRNA gene region. Modified from refs 2, 3 and 13. The arrow indicates the direction of transcription. The table shows the production of T4 tRNAs by various T4 tRNA deletion mutants. The size of deletions is taken from Wilson *et al.*⁵. *The size of $\Delta 105$ is unknown but on the basis of the tRNA pattern of the mutant it should be about the size of $\Delta 8$.



Mutant	Size of deletion base pairs	tRNA species									
		1	2	Arg	Ileu	Thr	Ser	Pro	Gly	Leu	Gln
<i>psu_b</i> $\Delta 64$	5820 $\pm$ 350										
<i>psu_b</i> $\Delta 33$	4150 $\pm$ 450	-	-	-	-	-	-	-	-	-	-
<i>psu_b</i> $\Delta 119$	2070 $\pm$ 190	+	+	+	-	-	-	-	-	-	-
<i>psu_b</i> $\Delta 27$	1330 $\pm$ 100	+	+	-	-	-	-	-	-	+	+
<i>psu_b</i> $\Delta 105$	~ 200*	+	+	+	-	-	-	-	+	+	+
<i>psu_b</i> $\Delta 8$	200 $\pm$ 30	+	+	+	+	+	-	-	-	+	+

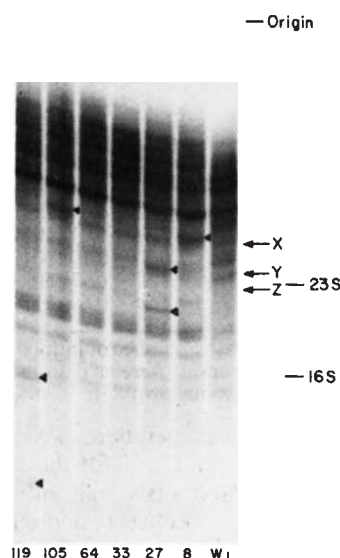


Fig. 2 Autoradiogram of acrylamide gel separation of primary transcripts of T4 DNA with intact or deleted tRNA genes. 5.5 μ g DNA from wild type (WT) T4 and *psu_b* deletion mutants $\Delta 119$, $\Delta 105$, $\Delta 64$, $\Delta 33$, $\Delta 27$ and $\Delta 8$ were transcribed by 20 μ g RNA polymerase in 100 μ l reaction mixtures containing 40 mM Tris-HCl pH 7.9, 10 mM MgCl₂, 7 mM 2-mercaptoethanol, 250 mM KCl, 0.25 mM each of CTP, GTP and UTP and 0.125 mM of α -³²P-ATP (0.4 Ci mmol⁻¹, Amersham). The synthesis was initiated after 10 min preincubation at 37°C by addition of nucleoside triphosphates. After 15 min at 37°C, 5 μ g DNase I (Worthington) and 25 μ g carrier RNA were added. The RNA was extracted with phenol, precipitated with alcohol, dissolved and fractionated by electrophoresis on 40 $\times$ 20 cm composite gel (2.2% acrylamide and 0.5% Agarose)¹⁴ in Tris-borate buffer containing 0.2% SDS at 150 V for 18 h. *E. coli* 23S and 16S [³²P]rRNA were run separately as reference markers. DNA-dependent RNA polymerase was purified from *E. coli* MRE 600 to DEAE-cellulose column step (modification II¹⁵) followed by two successive glycerol gradient centrifugations in 0.5 and 0.05 M KCl and phosphocellulose column chromatography in 50% glycerol¹⁶.

polymerase and the RNA products were fractionated by electrophoresis on composite acrylamide Agarose gels. Figure 2 shows that three high molecular weight RNA species (X, Y and Z) which are present among the transcripts of wild-type DNA are missing from the transcripts of the deletion mutants' DNA. New RNA species, whose positions in the gel are indicated by arrows, appear among the transcripts of the DNA of mutants *psu_b* $\Delta 8$, *psu_b* $\Delta 27$, *psu_b* $\Delta 105$ and *psu_b* $\Delta 119$. The positions of the new bands relative to bands X, Y, and Z of the wild type are correlated with the size of corresponding deletions in template DNA (Fig. 1). For example, the two short deletions, $\Delta 8$ and $\Delta 105$ (0.2 kilobases) cause production of new RNA transcripts which are only slightly smaller than X RNA of the wild type. Two deletions of intermediate size, $\Delta 27$ (1.33 kb) and $\Delta 119$ (2.07 kb) produce two new transcripts each, the longer deletion resulting in the appearance of shorter RNA molecules. Finally, the two long deletions $\Delta 33$ and $\Delta 64$ do not result in the production of any new RNA bands. It is reasonable to suggest that the new species appearing among the transcription products of the deletion mutants represent RNA molecules synthesised on the tRNA region shortened by the deletions.

RNA bands X, Y and Z from the gel of Fig. 2 were extracted, incubated with the 100,000g supernatant fraction (S-100) of *E. coli* and the digests analysed by electrophoresis on a two-layer 5%/10% polyacrylamide gel. Figure 3 shows that bands X (Fig. 3a), Y and Z (Fig. 3b) are all processed into several identical stable RNA species which migrate to the 4S region of the gel. This apparent identity demonstrates that bands X, Y and Z contain overlapping sequences and are thus transcribed from the same DNA region.

To determine which of the T4 tRNA sequences are present in the high molecular weight transcripts described above, we compared the stable RNAs obtained from band X with the products of S-100 processing of whole unfractionated RNA made on DNA containing the intact or deleted tRNA region. The deletions used in this experiment remove different T4 tRNA genes (Fig. 1) and should result in disappearance of different tRNA species. The cleavage products were first fractionated by electrophoresis on 10% acrylamide gel. The 4S regions of the gel were then cut out and applied on to 20% acrylamide gel slabs for the second dimension electrophoresis (Fig. 4). A comparison of the stable RNA patterns shows that at least five S-100 cleavage products (marked 1 to 5 in Fig. 4) are common to band X and the unfractionated transcript of wild-type T4 DNA, and that deletions result in the disappearance of spots corresponding to different stable RNAs.

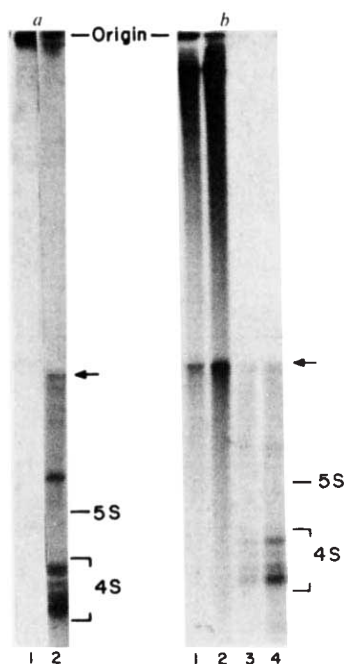


Fig. 3 Autoradiogram showing the formation of stable RNA from isolated precursor bands X (a), Y and Z (b). RNA bands X, Y and Z (Fig. 2) were eluted from the gel and incubated for 90 min at 37 °C with *E. coli* S-100 supernatant (5 mg ml⁻¹). The cleavage products were analysed by electrophoresis on a two-layer 5%/10% polyacrylamide gel (40 × 20 cm) in Tris-borate buffer¹⁴ with 0.2% SDS (300V, 12 h). Slots 2(a), 3 and 4(b) show the cleavage products of bands, X, Y and Z respectively. Slots 1(a), 1 and 2(b) show isolated uncleaved bands. The arrow indicates the boundary between 5% and 10% acrylamide concentrations.

One can see that stable RNA species 1 and 3 are present in the case of all the deletions used except $\Delta 119$. From the data of Fig. 1 and known lengths of T4 tRNAs³ one can conclude that species 1 is tRNA^{Leu} and species 3 is tRNA^{Gln}. Stable RNA species 2 which is affected by all four deletions used seems to be tRNA^{Pro} and stable RNA species 4 which is affected by all the deletions except $\Delta 105$ is tRNA^{Gly}. Stable RNA species 5 which appears in all cases except deletion $\Delta 27$ seems to be tRNA^{Arg}. From these results we conclude that high molecular weight band X RNA is indeed transcribed from the tRNA region of T4 DNA.

In addition to tRNA species 1 to 5, the cleavage products of band X RNA include several stable RNA species which are not seen among tRNAs produced from unfractionated wild-type T4 DNA transcripts. These RNA species may represent promoter-distal sequences which should be less abundant in a

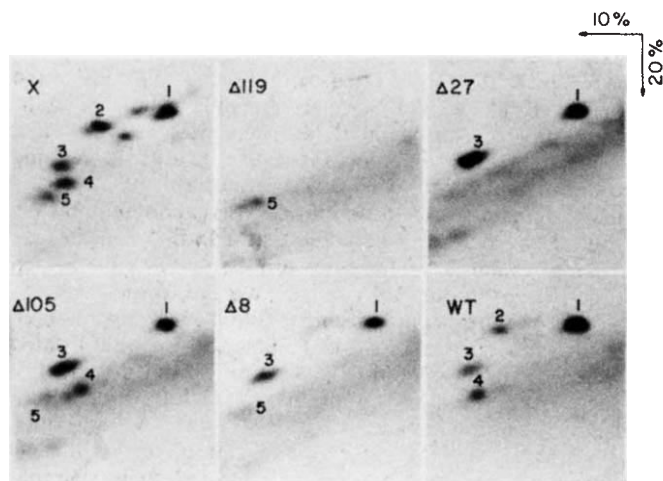


Fig. 4 Two-dimensional electrophoresis of stable RNA products of isolated precursor band (X) and unfractionated transcripts of DNA with intact (WT) or deleted tRNA genes. The conditions of RNA synthesis, isolation of band X RNA, S-100 processing and electrophoresis on a 10% polyacrylamide gel were as described in Figs 2 and 3. 4S regions of the 10% gel were cut out and applied on to 20% polyacrylamide slabs (15 cm long) for electrophoresis at 200 V for 14 h in Tris-borate buffer¹⁴.

complete unfractionated mixture of transcripts. As can be seen from Fig. 2, a considerable fraction of incorporated radioactivity is present in the background material which is DNA-dependent and RNase sensitive. This material seems to represent randomly terminated RNA chains which should be enriched by promoter-proximal sequences. Indeed, tRNA^{Gln}, tRNA^{Leu}, tRNA^{Gly} and tRNA^{Pro} (stable species 3, 1, 4 and 2) which are the most prominent on stable RNA pattern of wild-type transcript have proximal position on the genetic map of tRNA region (Fig. 1) while tRNA^{Arg} (stable species 5) whose gene is situated much further in the direction of transcription is not seen on stable RNA pattern of unfractionated wild-type transcripts. Yet, this promoter-distal tRNA (tRNA^{Arg}) appears on the stable RNA patterns of deletion mutants and is most prominent in the case of the longest deletion $\Delta 119$, a fact that should be expected since deletions must bring distal sequences closer to the promoter.

The T4 tRNA polycistronic precursor (band X RNA) is one of several high molecular weight transcripts made *in vitro* on T4 DNA. From its position in the composite gel relative to rRNA markers (Fig. 2) it seems to be about 5 kb long. Since the electrophoresis was carried out under conditions which preserve RNA secondary structure it is possible that the precursor is actually much larger.

The T4 tRNA genes are clustered within a distance of 2.5 kb in T4 DNA. The closest known genes on the two sides of this region are gene *ipI* (internal protein) and *e* (endolysin) which are about 6 kb apart and are transcribed from right to left² (Fig. 1). The following facts are known about the expression of this T4 DNA region^{6,8-11}: *ipI*, *e* and tRNA genes are transcribed from early promoters which are recognised *in vitro* by *E. coli* RNA polymerase; *ipI* and tRNA genes are immediate early genes and *e* is a delayed early gene since chloramphenicol inhibits the expression of *e in vivo*; *ipI* is situated very close to its promoter while *e* is located as far as about 6,000 nucleotides away from its promoter. These observations allowed Black¹² to suggest that genes *ipI* and *e* can be transcribed as a polycistronic mRNA. The T4 tRNA precursor described in the present report may well cover both *ipI* and *e* genes although it is not yet proved. In any case, the precursor molecule is long enough to allow us to suggest that the tRNA genes are cotranscribed either with *ipI* or with *e*. The tRNA genes are located very close to gene *ipI* (about 0.5 kb)² and therefore the tRNA

sequences will be situated at the 5' end of the polycistronic transcript (band X).

The two other tRNA precursor bands (Y and Z) are about the size of 23S rRNA (3.2 kb). Since RNA bands Y and Z contain the same promoter-proximal tRNA sequences as the longer RNA band X, their different size must be due to missing sequences at the 3' end. This may be the result of an earlier specific termination of transcription. We cannot completely rule out, however, the possibility that bands Y and Z are derived from band X by traces of processing nucleases which might be present in our RNA polymerase preparations.

The tRNA polycistronic precursor (band X) is long enough to include the sequences of the two stable RNA species located at the end of tRNA operon (Fig. 1). Yet, we did not identify these RNA species among the products of S-100 processing of band X or of unfractionated mixture of T4 DNA transcripts. Although some bands of the expected molecular weight have been observed, they were not reproducible and we believe that these RNA species are not completely stable under the processing conditions used.

The isolation of the polycistronic T4 tRNA precursor makes the T4 tRNA operon a promising system for the study of the mechanism of T4 gene expression and the maturation of tRNA.

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Unusual segmental flexibility in a region of tobacco mosaic virus coat protein

ASSEMBLY of tobacco mosaic virus (TMV) is known to occur by threading in the RNA on the inner side of the growing hollow rodlet of viral coat protein, and is thought to involve a pre-assembled double disk as an intermediate¹⁻³. Given the structure of the subunit⁴, such a mechanism requires that it be possible to move the segment of the polypeptide chain which separates the nucleic acid groove from the lumen of the cylinder so that it is out of the way in the assembly process. Evidence suggesting that the segment in question has some flexibility comes from X-ray diffraction studies of the virus and its components. In the intact virus studied by the Heidelberg group this segment (residues 102-112) apparently exists largely as an α -helix (the V-helix)⁴; on the other hand, in the crystals of the isolated coat protein disk studied by the Cambridge group, residues 102-112 and adjacent segments (88-100, 112-114) do not give a well-defined electron density, reflecting a high degree of disorder⁵. However, X-ray evidence is inconclusive on this point, as it does not distinguish between thermal disorder indicating flexibility and static disorder indicating irregular

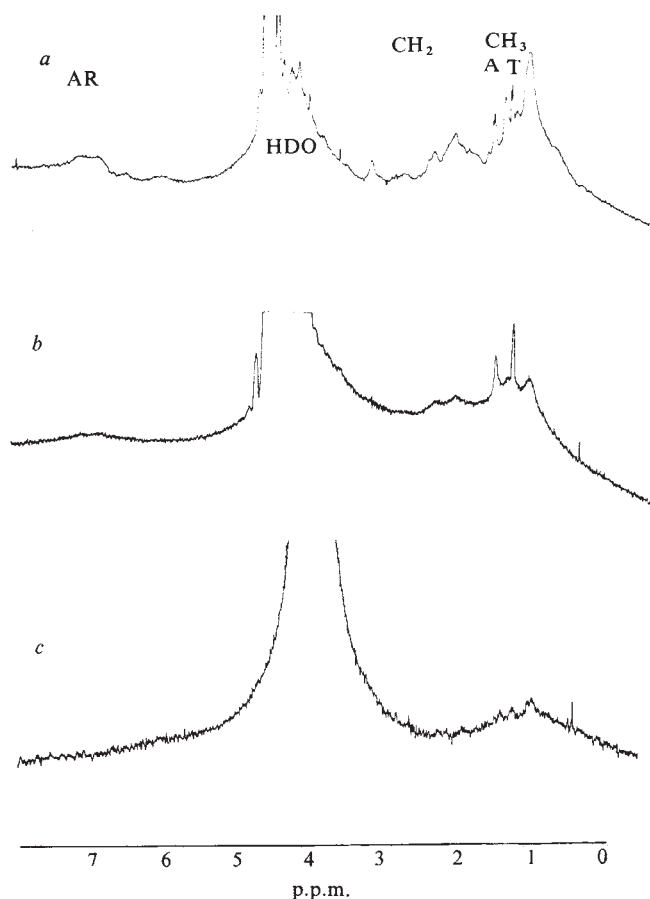


Fig. 1 ^1H NMR spectra of TMV coat protein at 360 MHz. Protein concentration 10 mg ml^{-1} in phosphate buffer $\Gamma/2 = 0.1$, temperature 27°C , pH as indicated. a, 4S particle, pH 8.5; b, disk (19-22S), pH 7.1; c, helix ($\geq 30\text{S}$), pH 6.0. Chemical shifts (δ) in p.p.m. from internal DSS. AR = Aromatic envelope; CH_2 = methylene envelope, CH_3 = methyl envelope; T = threonine doublet, $\delta = 1.17$ p.p.m.; A = alanine doublet, $\delta = 1.42$ p.p.m.

packing of the segment in the crystal. de Wit *et al.*^{6,7} have pointed out that there must be internal mobility in the TMV coat protein, based on the results of ^{13}C NMR of various TMV protein aggregates. Unfortunately, their results do not show whether or not there is flexibility in a particular segment of the protein. In an effort to distinguish between the thermal disorder and the static disorder in the V-helix segment, we describe here our investigation of the problem by high-resolution proton nuclear magnetic resonance (NMR) studies, and report a flexibility due to thermal motional disorder.

The 360-MHz ^1H spectrum of the coat protein (4S particle of TMV Vulgare), average molecular weight (MW) 52,000, as determined by sedimentation velocity on samples used in the NMR studies, is shown in Fig. 1a. The spectrum has several striking features. The aromatic region is surprisingly ill-resolved for a protein of this size, suggesting a relatively well-packed hydrophobic core (ref. 8 and A. Bloomer, personal communication). The aliphatic region is also relatively broad, as might be expected for a protein with a high helical content and tight packing. However, several sharp peaks occur in the 1.1-1.5 p.p.m. region downfield from DSS. Most impressive are two sharp doublets at 1.17 and 1.42 p.p.m. The relative sharpness of these lines persists as the effective molecular weight of the coat protein is increased by polymerisation to 8S particles, the 19S disk (average MW $\sim 650,000$, Fig. 1b) and to some extent even the RNA-free helix ($> 30\text{S}$, MW $> 10^6$, Fig. 1c).

There is an obvious possibility that the sharp lines originate from a small molecular weight contaminant. In protein NMR,

this possibility can sometimes be proved, but seldom rigorously ruled out. Indeed, a third sharp doublet at about 1.3 p.p.m. occurs in some TMV preparations and is readily shown to be a contaminant by the fact that its intensity can be markedly reduced by prolonged dialysis. This is not the case with the two peaks at 1.17 and 1.42 p.p.m. Their absolute and relative intensities are the same in many preparations of TMV Vulgare and several mutants, made in various laboratories, and is not altered by dialysis. Their T_1 is of the same order of magnitude as the T_1 of other protein peaks (compare results below), whereas the T_1 of the known contaminant is much longer (~ 2.4 s). The relative sharpness of these lines is lost on denaturation of the protein, whereas that of the contaminant is not. Even though the evidence is circumstantial, we believe these peaks to represent an integral part of the protein.

From comparison of chemical shifts and coupling constants we conclude that peak 1, $\delta = 1.17$, $J \approx 6.1$, is due to the methyl groups of threonines ($\delta \approx 1.2$, $J = 6.0$ – 6.6) and peak 2, $\delta = 1.42$, $J \approx 6.8$, to the methyl groups of the alanines ($\delta \approx 1.41$, $J = 6.8$ – 7.2). Alternative possibilities, such as unusually shifted methyl groups of valine, leucine or isoleucine, can be excluded by the multiplet structure and double resonance. Peak 1 is found to couple to an α -CH at 4.32 p.p.m., and peak 2 to one at 4.49 p.p.m., whereas the valine, leucine and isoleucine methyl groups couple to α -CH groups in the region of 1.5–3 p.p.m. The relative areas of the two peaks are approximately the same, and comparison with the area of the major peaks in the region 0–3 p.p.m. suggests that they represent three or four threonine and alanine residues each. It is noteworthy that this approximates the threonine and alanine content (four and three, respectively) of the V-helix segment (Ala 100 to Arg 112).

Direct proof that at least some of the sharp lines originate from the V-helix segment comes from an examination of a series of mutants with point amino acid substitutions in the segment. Figure 2a shows a comparison of the spectra of the 4S particle of TMV Vulgare, and Fig. 2b the mutant Ni 725, in which Thr 107 is replaced by methionine and Ileu 129 by threonine. Both tracings are obtained by resolution enhancement processing^{9,10}, so that the broader components are filtered out and only the sharper components remain. As Vulgare contains no methionine, the assignment of the sharp peak at 2.02 p.p.m. to the methionine methyl group in Ni 725 (ref. 11) is unequivocal. It should be noted that the peak occurring at 2.02 p.p.m. in the tracing in Fig. 2b is actually a superposition of a broader unassigned component present in both spectra, and a sharp methionine peak, which is readily identified in a Hahn-Spin Echo T_2 sequence ($1/\pi T_2 \sim 4.5$ Hz). Less certain are the assignments in the threonine region, as the threonine content of the two proteins is identical. It is probable that the line at 1.2 p.p.m. present in *a*, but not in *b*, represents Thr 107, and the line at 1.2 p.p.m. present in *b*, but not in *a*, represents Thr 129.

An estimate of the areas of the sharp components¹⁰ in both spectra also suggests that the peptide segment which shows signs of unusual mobility is longer than the proposed V-helix. Additional peaks with long T_2 values occur at 0.8 p.p.m., 1.12 p.p.m., 1.28 p.p.m., 1.35 p.p.m. and 1.6 p.p.m. Except for the alanine and threonine peaks, only four small peaks of Pro 102 and Leu 108 would be expected in this spectral region if only the V-helix contributed the narrow lines. If it is assumed that the entire segment Asp 88–Arg 112 is mobile, the resolution enhanced spectrum predicted from the amino acid composition is in good agreement with that observed. The possibility remains, however, that residues from other segments also contribute to this spectrum and this is being investigated using additional mutants.

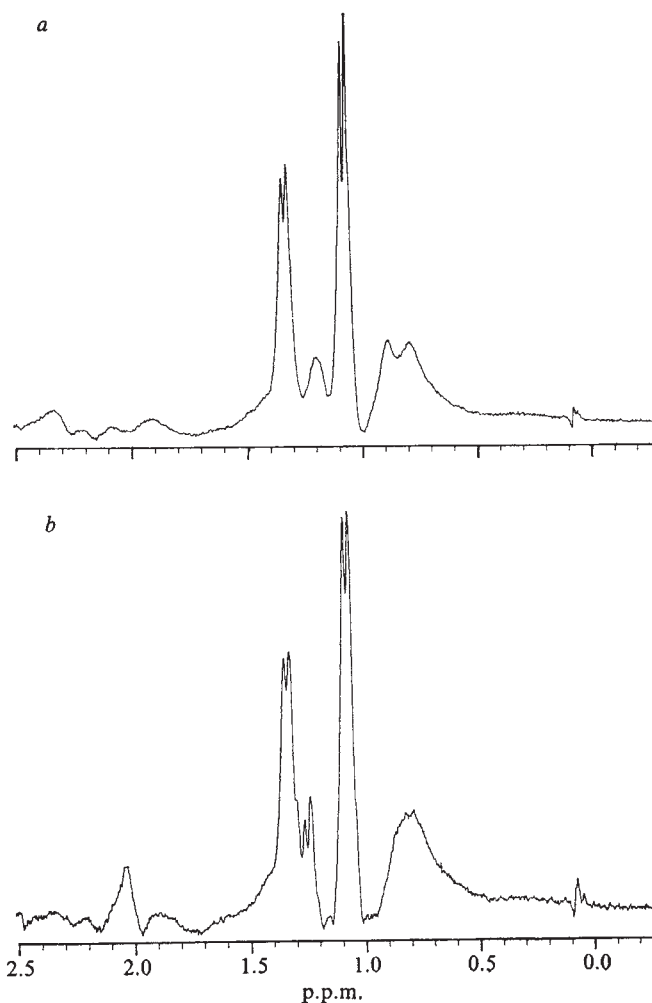
Relaxation measurements on the virus coat protein provide further evidence for the interpretation that the protein consists of a relatively compact core containing all the aromatic and most of the hydrophobic aliphatic side chains, and a relatively short, unusually mobile segment.

Our best estimates for the proton relaxation in the aliphatic region are $NT_1 = 600$ – 700 ms, $1/\pi T_2$ 10–20 Hz for the broad

components and $NT_1 = 700$ – 900 ms, $1/\pi T_2$ 2–4 Hz for the sharp components. An additional, still broader component for which relaxation measurements have not yet been possible may also exist in the spectrum. Neither of these pairs of measurements can be accounted for by a simple rigid sphere model of relaxation. The relaxation behaviour of the broad component can be approximated by using the 3-state model for internal rotation developed by Woessner¹², assuming a correlation time $\tau_c = 2 \times 10^{-8}$ s for the overall diffusion of the protein particle, and an average internal correlation time $\tau_i \sim 10^{-9}$ s for methyl group rotation. However, the model does not account for the behaviour of the sharp component. The theoretical treatment developed by King and Jardetzky¹³ was therefore used to analyse the data for this component. The best fit was obtained using a three-parameter 'Wobble-Woessner' model¹⁴ with time constant $\tau_c = 2 \times 10^{-8}$ s for rotational diffusion, $\tau_s \approx 10^{-9}$ s for a $\theta \approx 90^\circ$ segmental wobble and $\tau_{sc} \approx 5 \times 10^{-10}$ s for a 3-state side-chain rotation. The failure of several alternative models to account for the data leads us to believe that the model chosen is a fair approximation to the nature and the rates of the motions involved.

These NMR experiments and others in progress strongly support the notion that the disorder observed in the V-helix segment of the coat protein crystals is of a thermal motional nature. Random coil segments in nucleic acid binding proteins have been noted by Bradbury *et al.*¹⁵ in the case of histones. In our experiments, the unusual mobility is not observed by either X-ray or NMR in the intact virus. This suggests that the V-helix

Fig. 2 Resolution enhanced ^1H NMR spectra of the 1–2 p.p.m. region, TMV coat protein (4S particles) at 360 MHz. *a*, TMV Vulgare; *b*, TMV mutant Ni 725. Conditions as in Fig. 1a.



segment acts as a trap door which is open in the RNA-free protein and closed when the nucleic acid is in place.

The experiments reported here are also of interest in another connection. It has recently been claimed¹⁶ that the relaxation in ¹H spectra of globular proteins is dominated by spin diffusion and provides little information on internal motions. This might prove to be true in some cases, but the differential relaxation effects observed here provide a clear example of a protein for which it is not true.

The resolution enhancement processing reported in Fig. 2a and part of the relaxation measurements were carried out on the 360 MHz instrument of the Stanford Magnetic Resonance Laboratory supported by Grant RR00711 from the NIH and Grant GR23633 from the NSF. O.J., on leave from Stanford University, is recipient of a US senior scientist award of the Alexander von Humboldt Foundation, and acknowledges support from NIH Grant GM 18098 and NSF Grant PCM 75-02814. D.V. acknowledges support from a grant of the Deutsche Forschungsgemeinschaft VO 246/1+2. K.A. is on leave from Kyoto University and is a recipient of a fellowship from the Alexander von Humboldt Foundation. The authors thank H. J. Torff for amino acid analysis, Miss U. Gallwitz for technical assistance, and Dr P. Bray and N. Wade-Jardetzky for verifying the findings reported here in a series of experiments to be reported subsequently.

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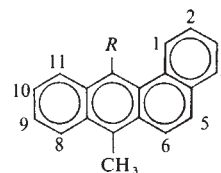
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Multiple regions of metabolic activation of carcinogenic hydrocarbons

SEVERAL lines of evidence have strongly implicated the bay region *anti*-diolepoxide of benzo(a)pyrene (BP) (Fig. 1) as the principal metabolically activated form of this potent environmental carcinogen¹. A bay region is defined simply as a molecular region such as the 4,5-positions of phenanthrene. Recent studies indicate the bay region diolepoxides of 3-methylcholanthrene (3-MC) (refs 2, 3), benz(a)anthracene^{4,5}, 7-methylbenz(a)anthracene (MBA) (ref. 6), 7,12-dimethylbenz(a)anthracene (DMBA) (refs 7, 8), and dibenz(a,h)anthracene⁹ as the ultimate carcinogenic forms of these hydrocarbons. (In

the cases of 3-MC, 7-MBA and 7,12-DMBA there is evidence for an additional benzylic hydroxyl group.) Jerina and Daly¹⁰ suggest, on the basis of simple molecular orbital calculations, that the bay region diolepoxides of carcinogenic polycyclic arenes are generally distinguished by exceptional reactivity and are the ultimately active forms of this class of carcinogen. We report here evidence for metabolites other than bay region diolepoxides as the active forms of at least some carcinogenic hydrocarbons.



MBA: R = H

DMBA: R = CH₃

Metabolic formation of the diolepoxides is believed to proceed by a sequence involving epoxidation, hydration to a *trans*-dihydrodiol, and a second epoxidation (Fig. 1). As epoxidation catalysed by the microsomal mixed function oxidases generally fails to occur at substituted aromatic ring positions¹¹, it is anticipated that carcinogenic activity should be blocked by substitution in the ring expected to undergo metabolic conversion to a reactive diolepoxide. To test this prediction, we investigated the carcinogenic activity of a series of methyl and fluoro derivatives of BP, MBA, and DMBA substituted in the ring believed to undergo metabolic activation. Our results are summarised in Table 1.

Methyl substitution in the 7- and 10-positions of BP dramatically altered the carcinogenic activity of the parent hydrocarbon. A single intramuscular injection of 1.0 or 2.5 mg of BP induced local sarcoma in 100% of the male Long-Evans rats tested.

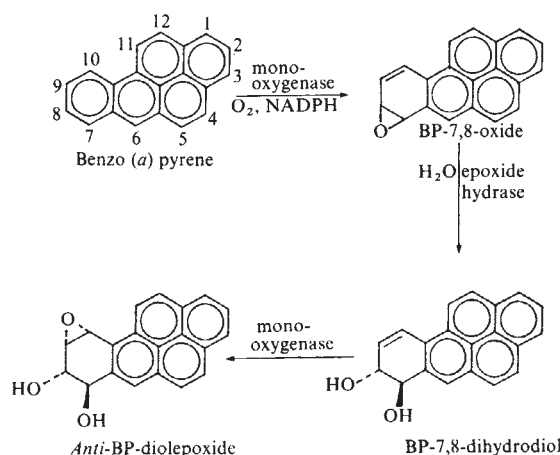


Fig. 1 Metabolic activation of BP catalysed by the microsomal mixed function oxidases.

In contrast, administration of 2.5 mg of 7,10-dimethyl-BP failed to elicit a carcinogenic response in 100% of animals tested. 10-Monomethyl-BP at similar dosage exhibited very weak activity (one-sixteenth positive), which may not be significant. However, 7-methyl-BP retained a high level of activity, a dose of 2.5 mg inducing sarcoma in seven out of eight rats (88%), equivalent at this dosage to that of the carcinogen dibenz(a,h)anthracene. At lower dosage (1 mg)

Table 1 Induction of sarcoma in male Long-Evans rats

Compound	Dose (mg)	Rats with sarcoma		Tumour induction time (d)	
		No.	%	Range	mean $\pm$ s.d.
BP	2.5	7/7	100	48-175	124 $\pm$ 16
	1.0	8/8	100	88-224	140 $\pm$ 42
7-CH ₃ -BP	2.5	7/8	88	115-187	152 $\pm$ 28
	1.0	4/7	57	161-222	187 $\pm$ 27
10-CH ₃ -BP	2.5	1/6	17	313	—
7-10-(CH ₃) ₂ -BP	2.5	0/8	0	—	—
DMBA					
4-CH ₃ -DMBA	2.5	8/8	100	52-97	79 $\pm$ 15
2-CH ₃ -DMBA*	2.5	0/16	0	—	—
3-CH ₃ -DMBA*	2.5	0/8	0	—	—
1-F-DMBA	2.5	0/8	0	—	—
2-F-DMBA	2.5	0/8	0	—	—
4-F-DMBA	2.5	5/8	63	110-128	123 $\pm$ 7
8-F-DMBA	2.5	8/8	100	57-92	65 $\pm$ 12
11-F-DMBA	2.5	4/4	100	64-111	92
MBA					
4-CH ₃ -MBA*	2.5	0/8	0	—	—
2-F-MBA	2.5	4/8	50	134-231	174
6-F-MBA	2.5	8/8	100	91-131	104 $\pm$ 13
9-F-MBA	2.5	4/8	50	136-282	243

Male Long-Evans rats, 25 d old were injected deep in the thigh muscle with a solution of 1.0-2.5 mg of the compound dissolved in 0.5 ml of sesame oil. The experiment was ended at 9 months. At necropsy, a pool of oil identified the site of injection of the compound. The diagnosis of sarcoma was made from histological examination of paraffin sections of tissue stained with hematoxylin-eosin.

*Carcinogenic activity of these compounds has been previously reported^{14,15} and is included for comparison.

7-methyl-BP caused a 58% (4/7) tumour incidence, indicating it to be a less effective carcinogen than BP itself, which evoked a 100% tumour incidence in similar conditions.

Methyl substitution also had a profound effect on the carcinogenic activity of DMBA, in which the fused angular ring is postulated to undergo metabolic conversion to a bay region diolepoxide. Methylation in the 2- or 3-positions of this ring completely abolished activity, whereas methylation in the 4-position resulted in no detectable loss in sarcomogenic activity in the conditions and at the dosage tested (2.5 mg); 4,7,12-trimethylbenz(a)anthracene is unquestionably a highly potent carcinogen. Similarly, introduction of a fluorine atom into the 1- or 2-positions of DMBA completely abolished carcinogenic activity, and substitution of this halogen in the 4-position decreased but did not destroy activity. Fluorine substitution remote from the bay region in the 8- and 11-positions had a similar effect, lowering without abolishing the level of induction of sarcoma.

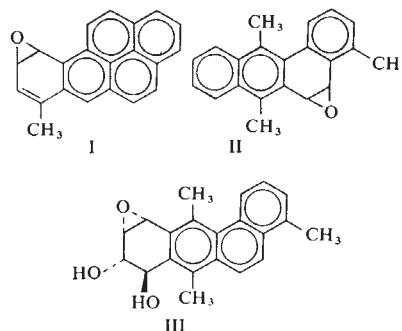
The effect of methyl and fluorine substitution in MBA did not entirely parallel that in DMBA. 4-Methyl-MBA, in contrast to 4-methyl-DMBA or MBA itself, exhibited no detectable carcinogenic activity, but 2-fluoro-MBA, in contrast to 2-fluoro-DMBA, proved to be a moderately effective carcinogen. Substitution of fluorine outside the region of presumed activation, in the 6- and 9-positions, had a similar effect, diminishing but not abolishing sarcomogenic activity.

These findings clearly demonstrate that introduction of methyl or fluoro groups into the ring of a polycyclic arene which is potentially capable of metabolic activation to a bay region diolepoxide may, but does not necessarily, abolish carcinogenic activity. Thus, 7,10-dimethyl-BP, 2-methyl-DMBA, 3-methyl-DMBA, 1-F-DMBA, 2-F-DMBA, and 4-methyl-MBA are inactive and 10-methyl-BP is extremely weak as a carcinogen, but 7-methyl-BP and 4-methyl-DMBA are very potent carcinogens, and 4-fluoro-DMBA and 2-fluoro-MBA exhibit diminished but still significant carcinogenic activity. This is strong evidence that metabolic activation by the mixed function oxidases must either occur relatively efficiently at substituted positions, or can occur at alternative molecular regions when reaction at preferred sites is blocked.

The former alternative seems improbable in the light of current knowledge of structural effects on microsomal oxidation. The latter alternative is the more intriguing, as it suggests multiple activated metabolites, the predominant metabolite being determined by which molecular regions are accessible for activation. If this is so, the problem of determining the nature of the metabolically activated forms of carcinogens becomes more complex than previously generally assumed.

Our results are consistent with those of other relevant studies. Thus, 7-methyl-BP was reported by Shear¹² to induce 38-62% skin carcinoma on repeated subcutaneous injection (10 mg) in mice. 3-Fluoro-MBA, not tested here, was found by Miller and Miller¹³ to be sarcomogenic on subcutaneous injection in both rats (93% incidence, 2 mg dose) and mice (61% incidence, 1 mg dose). 4-Fluoro-MBA in similar conditions was found to be too highly toxic to permit satisfactory evaluation of its carcinogenicity.

While clearcut evidence concerning the structures of the active metabolites of any of these compounds is lacking, the most probable candidates are arene oxides in the substituted ring (for example, I), K-region arene oxides (such as II), or non-bay region diolepoxides (III). Intermediates of these types are all anticipated to be capable of alkylation of nucleic acids. Research is in progress to determine which, if any, of these structural types are actually involved.



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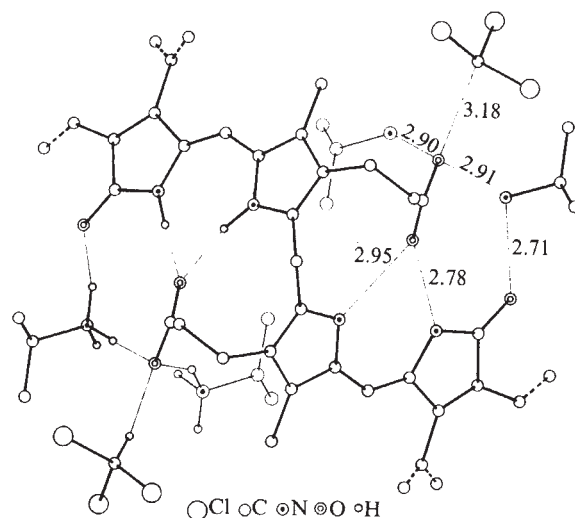


Fig. 2 Structure of the bilirubin-isopropylamine-chloroform complex. Hydrogen bonds are indicated by fine lines, the hydrogen atoms being pictured only in half of the complex (on the left). Atoms affected by disorder are at the outer end of the dashed bonds.

Structure of di-isopropylammonium bilirubinate

BILIRUBIN is the end product of haem breakdown in the reticuloendothelial system^{1–3} and is excreted into the bile after conjugation with various carbohydrates^{3,4} and polypeptides⁵. Because of low solubility in aqueous media⁶ (due to strong intramolecular hydrogen bonds^{7–12}), almost all of the unconjugated bilirubin occurring in the circulating blood is tightly but reversibly bound to serum albumin^{13,14}. In the liver cell the pigment is also thought to exist associated with soluble cytoplasmic proteins functioning as intracellular carriers^{15–17}. Binding of bilirubin to albumin seems to occur through formation of salt linkages and hydrogen bonds¹⁸; charged residues of histidine, arginine and tyrosine are involved in the binding¹⁹ and at least one lysine group is in or close to the primary binding site²⁰. However, the crystal structure of the pigment^{11,12} shows the presence of only intramolecular hydrogen bonds (four $\text{NH} \cdots \text{O}$ and two $\text{OH} \cdots \text{O}$ bonds). Thus the introduction of an amino group-bearing compound in the crystal should provide a useful model of the binding of bilirubin to albumin and other proteins. We report here the X-ray crystal structure of a molecular complex of bilirubin (Fig. 1) with isopropylamine and chloroform. This complex is a salt of bilirubin through the carboxyl groups, that is, the di-isopropylammonium bilirubinate with two chloroform molecules of crystallisation (Fig. 2). This X-ray analysis represents the first structural description of bilirubin in salt form.

Crystals of the complex bilirubin-isopropylamine-chloroform in the molecular ratio 1:2:2 were obtained by keeping overnight a solution of isomerically pure bilirubin IX α (ref. 21) (1 mg

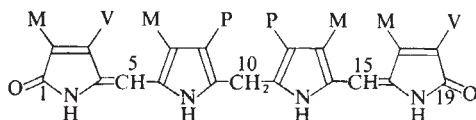


Fig. 1 Bilirubin IX α . M, methyl; V, vinyl; P, propionic chain.

ml⁻¹) in chloroform containing 1% isopropylamine (room temperature, dark). The complex crystallises in orange needles which are unstable to the air in the light. Specimens were sealed in Lindemann glass capillaries; preliminary X-ray photographs showed a strong decrease of intensities with increasing θ . Crystal data: $\text{C}_{41}\text{H}_{56}\text{N}_6\text{O}_6\text{Cl}_2$, monoclinic system, $a = 19.952$, $b = 12.867$, $c = 18.941$ Å, $\beta = 96.09^\circ$, $\rho_{\text{obs}} = 1.289$ (by flotation), $\rho_{\text{calc}} = 1.293$ g cm⁻³ for $Z = 4$, space group Ic or $I2/c$.

Reflections were recorded with a Philips PW 1100 automatic diffractometer using $\text{CuK}\alpha$ radiation and graphite monochromator, out to $\theta_{\text{max}} = 56^\circ$. A total of 3,130 independent reflections were collected; of these, 1,995 having $I \geq 1.5 \sigma(I)$ were considered as observed. A detailed description of the intensity measurement, the data treatment and the structure determination and refinement will be published elsewhere.

The structure was solved by direct methods, using the MULTAN²² program, with the assumption of space group Ic . The refinement led, however, to many unrealistic bond distances in the whole structure; moreover the terminal atoms of both vinyl groups of bilirubin gave very weak peaks on the Fourier maps. A disordered model was then assumed, by considering methyl and vinyl groups as randomly distributed about positions 2, 3, 17 and 18. Subsequent refinement was carried on in the centrosymmetric space group $I2/c$, by assuming a crystallographic twofold axis of symmetry running through the central methylene group (C10). After some refinement cycles, a further type of disorder was ascertained—the splitting of the *endo* vinyl group along two different orientations. A similar feature seems to be present even in the chloroform-bilirubin complex¹², and has been found in a related compound, the dimethyl ester of biliverdin IX α (ref. 23).

With the exceptions of very short bond lengths concerned with the disordered part of the structure, the molecular geometry turned out to be quite acceptable. Full-matrix anisotropic refinement (atoms affected by disorder, however, were treated as isotropic) led to an index $R = 9\%$ over the 1,995 observed reflections; the contributions of the hydrogen atoms (most of which were obtained from difference maps) were included in the F_2 calculations. The accuracy of the results is somewhat reduced by the large number of unobserved reflections in the explored range; the estimated standard deviations in C–C, C–N and C–O bond lengths are of the order of 0.01 Å.

Larger uncertainties, perhaps of the order of 0.1 Å, should be expected for the hydrogen atom positions.

Several structural features of the bilirubin bis anion do not differ substantially from those of the free acid in the crystal^{11,12}. Thus, the ridge tile shape is confirmed with a dihedral angle of 98° between the two oxodipyrromethene moieties which are both planar in a cisoid arrangement (syn-Z configuration). Values of bond lengths (Fig. 3) indicate an essentially double and single bond order for C4–C5 (and C15–C16) and C5–C6 (and C14–C15) respectively. Bond distances and bond angles are in good agreement with those determined in a single oxodipyrromethene compound²⁴. In the chloroform molecule and in the isopropylammonium ion values of bond lengths and angles are also in the normal range.

The carbon–oxygen bond lengths in the carboxyl group are approximately equal, corresponding to a carboxylate form. The nature of lactim–lactam tautomerism in bilirubin is controversial^{7,25}. In this study the values of carbon–nitrogen and carbon–oxygen bond lengths in the terminal rings, the experimental evidence in these rings of a hydrogen bound to the nitrogen atom, and the large value observed for the C4–C5–C6 bond angle—as a consequence of H···H repulsion in the oxodipyrromethene portion (see Fig. 3) allow the lactam form to be assigned.

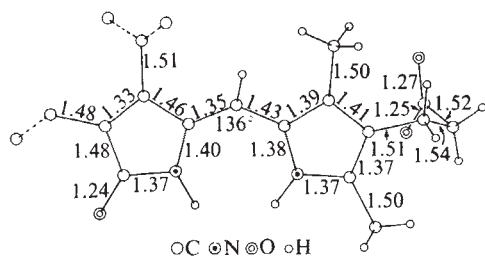


Fig. 3 The oxodipyrromethene moiety in the bilirubin–isopropylamine–chloroform complex.

The hydrogen bond pattern is of particular relevance (see Fig. 2). In contrast to bilirubin^{11,12}, in the present complex the conformation of the pigment molecule is stabilised by only two pairs of NH···O intramolecular bonds, each pair involving one carboxylate oxygen. Each of the remaining carboxylate oxygens is engaged not only in strong hydrogen bonds with two isopropylammonium ions but also in a weak CH···O hydrogen bond with chloroform. The shortest H bond of the complex involves the lactam oxygen atom and the third hydrogen of the isopropylammonium ion. Thus the structure of the di-isopropylammonium bilirubinate might be viewed as a model for bilirubin–protein associations.

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Protein transfer across microsomal membranes reassembled from separated membrane components

THE synthesis of secretory proteins is initiated on ribosomes in the cell cytoplasm and the first part of the polypeptide chain to appear is a short sequence termed the signal sequence¹. This signal is thought to direct the ribosomal complex to the endoplasmic reticulum (ER) membrane where the synthesis of the rest of the secretory protein is tightly coupled to its transfer across the membrane into the cisternal space. In all but one case, it is clear that the polypeptide chain is processed during transfer to remove the signal sequence^{2,3}. Little is known about the membrane proteins involved in conveying the growing polypeptide chain across the membrane. A classical approach to learning more about these proteins would be to dissect the microsomal membrane into inactive components that can be reassembled subsequently into a functional entity. This would allow the purification of components involved in protein transfer. Here we report the first such reassembly.

Messenger RNA coding for light-chain immunoglobulin (Ig) was translated in a wheat germ cell-free system in the presence of RNase-treated rough microsomes (RM_R) from canine pancreas⁵. The authentic Ig light chain (Li) was synthesised, together with a small amount of the light-chain precursor (p-Li) containing the signal peptide (Fig. 1, track 1). The authentic light chain was resistant to added proteases and its precursor was completely degraded (Fig. 1, track 2). Since added proteases degrade all but those proteins inside the vesicles, the transfer of the light-chain precursor into the vesicles is tightly coupled to the removal of the signal peptide. In the absence of membranes, no authentic light chain was synthesised (Fig. 1, track 3) and the precursor was completely degraded by added proteases (Fig. 1, track 4). The precursor synthesised in the presence of RM_R membranes was < 10% of the total Ig Li and could be increased by limiting the amount of membrane, and hence the number of sites, available for protein transfer. In all the experiments described the total membrane surface area was adjusted (using the phospholipid content of the membrane) to the same level, which, for RM_R membranes, was sufficient to transfer and process about 90% of the total Ig Li synthesised.

When RM_R membranes were washed with 0.5 M KCl, the resulting RM_{KK} membranes had almost lost the capacity to transfer and process the Ig Li. Precursor was synthesised together with only a small amount of the authentic light chain (Fig. 1, track 5), and it was completely degraded by added proteases (Fig. 1, track 6). The small amount of authentic light

chain was protected against digestion by protease and represented the residual transfer activity of the RM_{RK} membranes. Protein transfer and processing could be fully restored to RM_{RK} membranes by readdition of the salt extract (SE); the authentic light chain was synthesised together with some precursor (Fig. 1, track 7) but only the former was resistant to added proteases (Fig. 1, track 8). Treatment with high salt thus removes, reversibly, membrane components essential for protein transfer and processing.

The active components in the SE were characterised using a rapid, quantitative assay (see legend to Table 1) devised to facilitate their eventual purification. The assay determines the percentage of total protein synthesised that is resistant to added proteases because of transfer into vesicles. We used canine pancreatic mRNA in this assay since it codes mainly for secretory proteins and 40–55% of the protein synthesised is resistant to protease digestion. At least 80% of the pancreatic mRNA used in these experiments codes for secretory proteins (unpublished observations), so this level of resistance suggests that a fraction of the microsomal vesicles, containing newly-synthesised secretory proteins, are leaky to added proteases

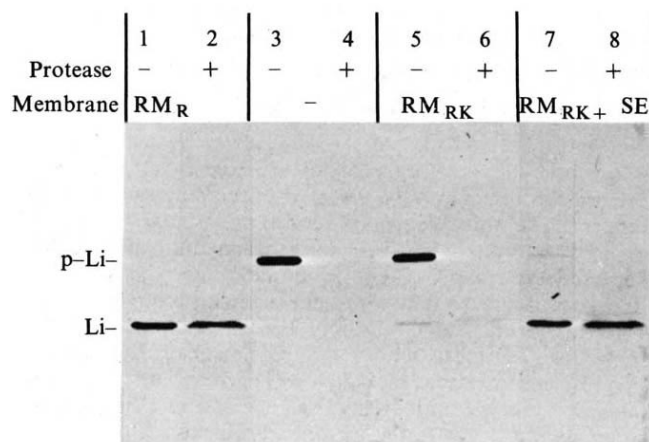


Fig. 1 Effect of KCl treatment of canine rough microsomes on the transfer and processing of light chain immunoglobulin. Rough microsomes (RM) from canine pancreas were prepared as described previously¹, and suspended to 1 mg membrane phospholipid ml^{-1} in 20 mM HEPES pH 7.5 (at 20 °C), 110 mM potassium acetate, 3 mM magnesium acetate and 2 mM dithiothreitol (microsomal buffer). $CaCl_2$ was added to 1 mM and micrococcal nuclease (Boehringer) to 10 $\mu g\ ml^{-1}$ followed by incubation at 20 °C for 15 min to remove endogenous mRNA. The nuclease was inhibited by chelating Ca^{2+} with EGTA added to 2 mM, the membranes washed by pelleting through 0.5 M sucrose, 20 mM HEPES pH 7.5 at 60,000g for 20 min at 4 °C and resuspended in microsomal buffer containing 1 mM EGTA. Aliquots of these RM_R membranes containing 50 μg of membrane phospholipid (90 μg protein) were used in each 100- μl assay. For the salt treatment, RM_R membranes were suspended in ice-cold 0.5 M KCl, 20 mM HEPES pH 7.5 to 10 mg membrane phospholipid ml^{-1} and pelleted as above. The pellet (RM_{RK} membranes) was resuspended in microsomal buffer and 50 μg membrane phospholipid (80 μg protein) was used in each 100- μl assay. In a parallel experiment, the sucrose cushion was omitted and the mixture centrifuged at 100,000g for 1 h at 4 °C. The supernatant salt extract (SE) was retained and 8 μg of protein was used in every 100 μl assay. The RM_R , RM_{RK} and SE preparations were frozen in liquid nitrogen and stored at -80 °C in small aliquots. The assay for transfer and processing has been described in detail previously¹. Briefly, mRNA was isolated from a MOPC 41 tumour secreting Ig light chain, and translated in a cell-free wheat germ system containing ³⁵S-methionine in the presence and absence of microsomal membranes. After incubation at 25 °C for 1 h, samples were either immunoprecipitated with antibody to light chain, or treated with proteases to determine the nature of the light chain transferred into the microsomal vesicles. Aliquots from both treatments were analysed by SDS-PAGE. The upper band is the precursor light chain (P-Li) and the lower band, derived from the precursor by proteolytic removal of the signal peptide, is the authentic light chain (Li) of immunoglobulin that would be secreted *in vivo*.

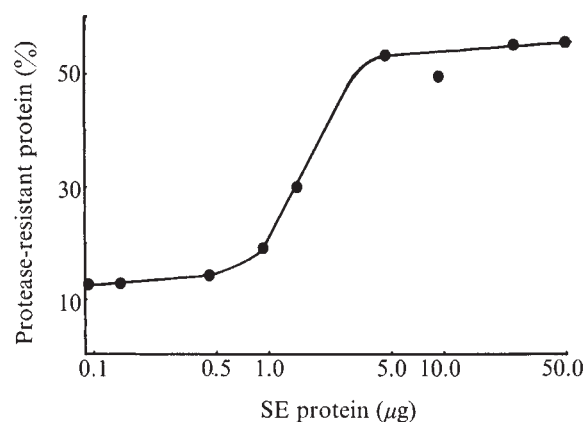


Fig. 2 Titration of the salt-washed microsomal membranes with the salt-extracted membrane proteins. Incubations were carried out as described in Fig. 1 and the quantitative assays as described in Table 1. The salt extract was concentrated by ultrafiltration using a Minicon with a B15 membrane (molecular weight cut-off = 15,000) and varying amounts were added to RM_{RK} membranes (50 μg membrane phospholipid per 100 μl assay).

(compare ref. 6). The percentage of leaky vesicles in any membrane preparation, however, was unaffected by the membrane treatments we used. RM_R membranes containing radiolabelled secretory proteins were subjected to these various treatments and 40–50% of the proteins synthesised were found to be resistant to added proteases. Membrane disruption is therefore not the cause of some of the results presented here.

RM_R membranes protected 39% of the total proteins synthesised and salt washing reduced this to 12%. Approximately half of the protection afforded by RM_{RK} membranes to pancreatic secretory proteins can be attributed to residual transfer activity (compare with Fig. 1, tracks 5,6 for the Ig Li); the other half is a feature of the assay and represents newly-synthesised proteins associated with membrane vesicles so as to allow only partial digestion by added proteases. This small amount of protein did not vary significantly from experiment to experiment and did not affect interpretation of the results. Readdition of SE to RM_{RK} membranes at the beginning of the incubation restored the original level of protection, whereas readdition at the end had no effect. In the absence of any other membranes, SE alone was unable to protect the secretory proteins (Table 1, experiments 1–5).

Proteins are the active components in the SE. Dialysis to remove small molecules did not impair its ability to restore transfer activity to RM_{RK} membranes, whereas heat or trypsin treatment destroyed it (Table 1, experiments 6–8). RNA does not seem to be an active component because the extract was derived from RNase-treated membranes. No phospholipids were detected in the extract.

The proteins which restore transfer activity to RM_{RK} vesicles are bound tightly to the membrane. By varying the amount of SE added to RM_{RK} membranes it was possible to increase the protection from 12 to 55% at saturating concentrations (Fig. 2). We estimated that 3.4 μg of SE protein had to be added to RM_{RK} membranes (containing 50 μg phospholipid) to saturate the membrane sites that rebind the SE proteins. From RM_R membranes (containing 50 μg phospholipid) we obtained 3.1 μg of SE protein. More than 90% of SE protein added to RM_{RK} membranes must, therefore, have been rebound. These data also indicate that RM_R membranes, as isolated, are nearly saturated with SE proteins.

The membrane proteins extracted with high salt are not solely responsible for protein transfer across microsomal membranes because the SE will not restore transfer activity to RM_{RK} membranes that have been trypsin treated, nor will it confer transfer activity on sonicated liposomes derived from

Table 1 Characterisation of the components in the salt extract that restore transfer activity to salt-washed microsomal membranes

Experiment	Membrane or lipid*	Treatment	Protease-resistant protein %
1	RM _R membranes	—	39
2	RM _{RK} membranes	—	12
3	RM _{RK} membranes	8 µg SE protein added at beginning of incubation	43
4	RM _{RK} membranes	8 µg SE protein added at end of incubation	4
5	—	8 µg SE protein	1
6	RM _{RK} membranes	8 µg SE dialysed SE protein	42
7	RM _{RK} membranes	8 µg heat-treated SE protein	11
8	RM _{RK} membranes	8 µg trypsin-treated SE protein	11
9	Trypsin-treated RM _{RK} membranes	—	5
10	Trypsin-treated RM _{RK} membranes	8 µg SE protein	5
11	Sonicated RM lipids	—	1
12	Sonicated RM lipids	8 µg SE protein	2
13	Sonicated egg phosphatidylcholine	—	3
14	Sonicated egg phosphatidylcholine	8 µg SE protein	3
15	RM _{RK} membranes	8 µg SE protein from SM membranes	13
16	SM membranes	—	11
17	SM membranes	8 µg SE protein	14

Incubations were carried out in the presence of ³⁵S-methionine as described in the legend to Fig. 1 except that 4 µg of canine pancreatic mRNA was added to each 100-µl assay instead of light chain mRNA. Unless otherwise stated, membranes, lipids and the salt extract were added at the beginning of the assay and the SE was derived from RM membranes. At the end of the 1-h incubation at 25 °C, the samples were cooled in ice and three aliquots taken for assay: In the first, 10-µl aliquot was sampled directly on to filter paper to determine the total protein synthesised; in the second, a 20-µl aliquot was treated with 80 µl of 2 mg ml⁻¹ proteinase K (Merck) in 50 mM HEPES pH 7.5 and incubated at 25 °C for 10 min, before a 50-µl aliquot was sampled on to filter paper; in the third, a 20-µl aliquot was treated with 80 µl of 2 mg ml⁻¹ proteinase K in 50 mM HEPES pH 7.5, 15 mM deoxycholate and incubated as in the second. All filter papers were treated according to the method of Mans and Novelli⁸. The proteinase K degrades nearly all of the proteins not inside the vesicle to such a small size that they do not precipitate on the filter paper when soaked in 10% TCA. The background ³⁵S-methionine bound to the filter paper after proteinase K treatment in the presence of deoxycholate, was always <5% of the ³⁵S in the total synthesised protein and was subtracted from the total counts and those obtained in the presence of proteinase K alone. These corrected counts were used to determine the percentage of protease-resistant protein [(³⁵S counts resistant to proteinase K/Total ³⁵S counts) × 100]. In the absence of RM_R, RM_{RK} and SM membranes, the total ³⁵S counts in the newly-synthesised proteins was typically 150,000 ± 20,000 c.p.m. In the presence of these membranes and irrespective of the various treatments, the total ³⁵S counts were between 90,000 and 120,000 c.p.m. Smooth microsomes were prepared by the method of Adelman *et al.*⁷ and were extracted with 0.5 M KCl as for RM_R membranes in Fig. 1. Dialysis of the SE was carried out against 2,000 vol 0.5 M KCl, 20 mM HEPES pH 7.5 at 4 °C for 15 h. Heat treatment was carried out on a 50-µl aliquot of SE (0.8 mg protein ml⁻¹) at 70 °C for 5 min. Trypsin treatment of RM_{RK} and SE were carried out by treating 50-µl aliquots with 1 µl of 1 mg ml⁻¹ trypsin and incubating at 25 °C for 20 min. Soybean trypsin inhibitor (1 µl of 5 mg ml⁻¹) was then added and the samples used directly in the assay. Controls using a mixture of trypsin and soybean trypsin inhibitor were shown to have no effect on any of the incubations. RM lipids were extracted from RM membranes using 20 vol CHCl₃-MeOH (2:1, v/v) and the organic phase removed under a stream of N₂ and then *in vacuo*. Egg phosphatidylcholine was purchased from Sigma. Lipids were suspended to 5 mg ml⁻¹ in 50 mM HEPES pH 7.5 by sonication for 5 × 2 min at <10 °C using a Branson sonifier with microtip set at 40 W. Phospholipids were assayed by the method of Bartlett⁹ and proteins by the method of Lowry *et al.*¹⁰.

* In all cases the samples contained 50 µg phospholipids.

RM lipids or egg phosphatidylcholine (Table 1, experiments 9–14). The membrane proteins in the SE are not found in ER membranes devoid of attached ribosomes *in vivo*. Smooth microsomal (SM) membranes, substantially freed of RM membranes, were extracted with 0.5 M KCl and this salt extract did not confer transfer activity on RM_{RK} membranes (Table 1, compare experiments 2 and 15). The SM vesicles were themselves unable to transfer secretory proteins (Table 1, experiment 16) and this inability was not due simply to a lack of the membrane proteins present in the SE from rough microsomes. Addition of this SE to SM vesicles did not result in protein transfer (Table 1, experiment 17).

The membrane proteins isolated by treating RM membranes with high salt are needed for the transfer of secretory proteins across the ER membrane. They can restore transfer activity to inactive, but intact, salt-washed vesicles and are normally bound tightly to the membrane. They are thus proteins bound to the cytoplasmic side of the microsomal membrane and probably do not span it; they may, however, be bound to proteins that do span the bilayer. Their location would suggest that they are involved in the binding of the signal peptide and associated ribosomal complex to the membrane. Other membrane proteins are necessary for protein transfer but they are more likely to be involved in events subsequent to binding.

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matters arising

Dynamic measurement of matter creation and Earth expansion

ITTER *et al.*¹ have attributed to me the argument that continuous creation of matter where matter is already most dense at a rate of $7 \times 10^{-18} \text{ g g}^{-1} \text{ s}^{-1}$ can account for the growth of the mass of the Earth ($6 \times 10^{27} \text{ g}$) in a time of $4.5 \times 10^9 \text{ yr}$. This is certainly numerically the case (ignoring the fact that the mass of the proto-Earth in the early stages would have been small and the creation rate correspondingly low). However, in the paper of mine which they quote², I pointed out (on page 54) that the observed upper limit on continuous creation (of Cohen and King³) of $4 \times 10^{-23} \text{ g g}^{-1} \text{ s}^{-1}$ seems to rule out such a process. In order to remove doubt, it should be pointed out that the continuous creation hypothesis for the origin of the Earth will only be plausible if the experiment described by Ritter *et al.*¹ disproves the results of that of Cohen and King³.

The continuous creation rate of $7 \times 10^{-18} \text{ g g}^{-1} \text{ s}^{-1}$ is that given by the condition that the Universe be in a steady state^{4,5}. It is indeed a remarkable coincidence that this creation rate can account for the Earth's mass with its known geological age (T_E); but it should be appreciated that what this coincidence means is that the timescale T_E is

$$T_E \approx 1/(7 \times 10^{-18}) \approx T_U \approx H^{-1} \quad (1)$$

where T_U is the 'age' of the Universe, which is believed to be of the order of the reciprocal of Hubble's parameter H . But the rate of expansion of the Earth at about 0.5 mm yr^{-1} , which is apparently observed⁶ and which is remarked on by Ritter *et al.*¹ (in connection with continuous creation) also has another possible explanation: as pointed out some time ago⁶, if one puts the radius of the Earth R_E into the Hubble formula one finds

$$R_E \approx HR_E \approx 0.5 \text{ mm yr}^{-1} \quad (2)$$

with $R_E \approx 6,370 \text{ km}$ and H equal to the cosmological value of $75 \text{ km s}^{-1} \text{ Mpc}^{-1}$ ($= 2.5 \times 10^{-18} \text{ s}^{-1}$). The coincidence, equation (2), seems to be at least equally as impressive as equation (1), and suggests that the expansion of the Universe may in some way be coupled to smaller systems⁷. If a system expands in this manner, whether as a response to continuous creation or some other process, the resultant change in the angular velocity of

the system should be open to test using the experiment described by Ritter *et al.*¹.

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Do compact radio sources contain non-relativistic plasma?

THERE has been considerable discussion^{1–3} of multifrequency polarisation measurements of compact variable radio sources. These measurements^{4,5} indicate that the synchrotron radiation emitted by compact sources has undergone no significant amount of Faraday depolarisation. It has been argued^{1–3} that these data imply that such radio sources contain no non-relativistic plasma. It is, therefore, concluded that radio source models^{6–8} which rely on plasma turbulence in a non-relativistic background plasma for particle acceleration are inapplicable.

We wish to point out first, that these limitations actually apply only to the presence of non-relativistic electrons, and that Faraday depolarisation observations are insensitive to the presence of protons or other massive particles whether they are relativistic or not. (Faraday rotation due to 'cold' protons is a factor $m_p^2/m_e^2 \sim 3 \times 10^6$ less than that due to non-relativistic electrons.) In fact, the observations are consistent with the non-relativistic proton density which is more than 10^4 times greater than the density of observed, relativistic electrons, and statements³ that the compact sources contain 'no non-relativistic matter' are, therefore, completely unjustified. Furthermore, the data of Wardle² seem to indicate source temperatures of the order of 10^{12} K . At this temperature the thermal electrons would be expected to be relativistic, as observed, but the thermal protons would be non-relativistic ($\gamma_p = E_p/m_p c^2 \sim 1.2$). Theoretical considerations also indicate⁹, in agreement with Wardle's observations, that 10^{12} K is a natural

upper limit to the proton temperature as pion production cooling acts as a natural thermostat mechanism and precludes higher temperatures.

It, therefore, seems likely that the internal energy state and dynamics of compact sources are dominated by a non-relativistic proton plasma. Thus, we also disagree with the previous contentions^{1–3} that those source models are inapplicable which invoke non-relativistic plasma turbulence as a particle acceleration mechanism. Some models⁹ rely on electron-supported electrostatic plasma turbulence, and the lack of non-relativistic electrons in compact sources does seem to rule out such theories (see ref. 3). However, models dealing with non-relativistic hydrodynamic turbulent acceleration⁸ (such as statistical Fermi acceleration), Alfvénic turbulent⁷ acceleration, or combinations thereof¹⁰ are consistent with observations, as these types of turbulence are all supported by the background non-relativistic proton plasma.

The absence of Faraday depolarisation of the radiation from compact radio sources cannot, therefore, be used to determine the internal energy state of such sources, and models of compact sources including non-relativistic plasma turbulent particle acceleration agree with observations.

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WARDLE REPLIES—I do not disagree with the above comments. The physical picture described by Christiansen *et al.*¹ seems to be very similar to that suggested by us^{2,3}, that

is, a thermal plasma at a temperature of about 10^{12} K.

Obviously, Faraday rotation measurements are not directly sensitive to protons. But if electrical neutrality is preserved, they do strongly limit the proton number density.

The observations tell us that there are very few non-relativistic electrons inside the radio-emitting region, and, therefore, that the acceleration mechanism must be extremely efficient. This is the fundamental constraint that must be satisfied by whatever acceleration process is invoked.

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Topography of random surfaces

SAYLES and Thomas have claimed that the topographies of natural and artificial random surfaces commonly possess isotropic spectra $G(\omega)$ which fall with spatial frequency ω according to the power law

$$G(\omega) = 2\pi k / \omega^2 \quad (1)$$

where k (which they call the 'topothesis') is a constant depending on the surface chosen, and α takes a universal value of 2.

They derive $\alpha=2$ from a theoretical argument based on the assumption that above any horizontal straight line the changes of surface height Δh_{12} and Δh_{23} along consecutive segments 1, 2 and 2, 3 of the line are statistically independent. But there is no reason *a priori* that this assumption should hold in practice. For a general α there will be correlation between the height change, even for line segments separated by an arbitrarily large distance. This is consistent with their principle that "a sample of finite length taken from such a surface will never, however long, completely represent its properties". Statistically isotropic surfaces which have no scale and whose height function is well defined but non-differentiable may have spectra of the form of equation (1) with any α in the range $1 < \alpha \leq 3$. Their geometric properties are discussed in detail by Mandelbrot² who calls them 'fractals' to emphasise that they can be considered as having fractional dimensionality

$$D = (7 - \alpha) / 2 \quad (2)$$

lying between 2 and 3. Sayles' and Thomas' argument is restricted to the 'Brownian' case $D=2.5$. In the general case their useful concept of topothesis is better defined as the length

$$T = k^{1/(3-\alpha)} \quad (3)$$

which is proportional to the horizontal distance, L , between two points on the surface whose connecting line has r.m.s. slope unity, $(J\langle(\Delta h)^2\rangle)^{1/2} = L$.

Experimental evidence is adduced to support $\alpha=2$ but the treatment of the data is procrustean. Their Fig. 2 combines measurements on 23 types of surface spanning nearly eight decades in wavelength. However, the points representing the individual types of surface cover much smaller ranges and inspection shows that when fitted with spectra of the form (1) their separate α vary from 1.07 to 3.03 (nicely covering the fractal range). Sayles and Thomas seem to have fitted each set of data to a prescribed spectrum with $\alpha=2$ thus determining the constant k , and then plot all the 'reduced' spectra $G(\omega)/k$ on the same graph. On a plot with axes $x = \log(2\pi/\omega)$, $y = \log G$ this amounts to taking raw data consisting of 23 short line segments with varying slopes scattered over the x, y plane and translating each one along y so that it lies as close as possible to the line $y=2x$, a procedure guaranteed to produce a better looking fit as the total range covered by the data increases.

Therefore the claim to have discovered a "common natural law underlying the phenomena" of surface topography cannot be justified or supported by the experiments they cite.

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SAYLES and THOMAS REPLY—We welcome the comments of M. V. Berry and J. H. Hannay¹ and would like to take the opportunity to clarify some of their points. We feel that their objection to our derivation is a misunderstanding caused by our invoking the theory of Wiener², as we do not require the changes in height to be statistically independent, only the heights themselves. An unambiguous outline of our derivation is discussed in the first paragraph of our letter.

Our Fig. 2 was, in fact, constructed by the technique Berry and Hannay suggest but this was through necessity and was not intended to appear procrustean. We could find no better and unambiguous technique for comparing the experimental points from the 23 different sources with our derived model. As most surface measurement techniques can only record information over approximately two decades of wavelength, we would expect a variation in α of the $\omega^{-\alpha}$ (where $\alpha=2$)

law to occur. Although we have no formal proof we consider our derived $\alpha=2$ as the mean condition and the variation of $1 < \alpha < 3$ suggested by Mandelbrot³ as being an upper and lower limit of possible variation. This implies that the probability of obtaining $\alpha \neq 2$ increases as the bandwidth reduces, which appears to be the case with our data. The longest spectrum we show, a supertanker hull plate, covers approximately four decades and although ω^{-2} is not the best fit within short segments, over the total range it does represent the best $\omega^{-\alpha}$ power law fit. Our Fig. 2 depicts all of what one might call the worst experimental points, many points which would fit an ω^{-2} law more closely had to be omitted to avoid unnecessary confusion.

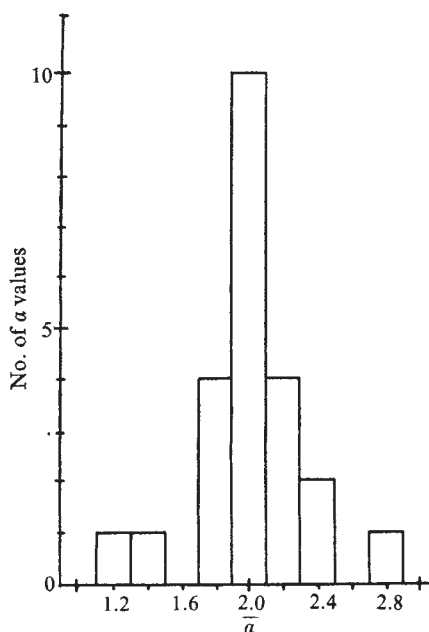


Fig. 1 Histogram of the values of α obtained from fitting a power law of the form $\omega^{-\alpha}$ to the 23 sets of experimental points of our previous Fig. 2 (ref. 1).

Without adding the points omitted from Fig. 2 we have replotted all the spectra as Berry and Hannay did, and the values of α are shown as a histogram in Fig. 1. Mandelbrot (personal communication) suggests that α should take a value of about 2.5, whereas we find from Fig. 1 (as Berry and Hannay must have done, although they failed to mention it), the mean value of α is 1.98 and, therefore, find no reason to change our claims.

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reviews

March of paradigms

P. B. Medawar

The Essential Tension: Selected Studies in Scientific Tradition and Change. By Thomas S. Kuhn. Pp.366. (University of Chicago Press: Chicago and London, 1978.) \$23; £12.95.

As a general rule, scientists are too busy with their research and often too practically minded by temperament to pay much attention to what philosophers or historians of science have to say about what they are or ought to be doing. When, therefore, a philosopher's views catch on — as Popper's have, and in recent years Thomas Kuhn's — it is a sure sign that he may have something sensible and helpful to say.

Kuhn's views first became known through his *The Structure of Scientific Revolutions* (University of Chicago Press, second edition, 1970). *The Essential Tension* is a little compendium of essays or contributions to learned journals which enlarge on or expound one aspect or another of his thought; as such, it makes a very useful and agreeably readable introduction to his philosophy. The "essential tension" to which Kuhn refers is between our cultural inheritance of doctrine and dogma and the occasional upheavals of opinion that inaugurate a new orthodoxy or network of received beliefs — a new "paradigm" in the terminology Kuhn has made popular.

A reader intending to use *The Essential Tension* as an introduction to Kuhn's thought would do well to begin with the essay entitled "Logic of Discovery or Psychology of Research". From this he will learn that the views of Kuhn and of Popper, so far from being wholly antithetical, have much in common: of course scientists think up hypotheses and propound theories to explain the goings-on of the natural world and of course they then try to find out if their ideas hold water. It is over the character of this critical process that the views of Popper and Kuhn diverge most widely. Kuhn sometimes writes as if he imputed to Popper the notion that the testing of an hypothesis is a kind of private transaction between the scientist and reality, as a result of which the hypothesis either remains on probation or is falsified. Kuhn's view is that a scientist's ideas are typically assayed by reference to the prevailing

"paradigm" — the prevailing "establishment" of received beliefs and current opinions. Ordinary or "normal" science, according to Kuhn, is for the most part a matter of puzzling, as far as possible within this framework of received opinion. Every so often, however, the extraordinary happens: the prevailing orthodoxy is found wanting and is supplanted rather rapidly by a new orthodoxy which constitutes a new paradigm. In a first-rate symposium devoted to the exposition and criticism of Kuhn's views (*Criticism and the Growth of Knowledge*, ed. I. Lakatos and A. Musgrave, Cambridge University Press, 1970) — a volume that a serious student of Kuhn's views should read as a companion to the present one — J. W. N. Watkins remarks that "Kuhn sees the scientific community on the analogy of a religious community and sees science as a scientist's religion": so that the supplanting of one paradigm by another might in the spirit of this parallel be thought of as a confused and anxious period marked by shrill cries of heresy and schism.

My own view is that although Kuhn's views are in many ways psychologically illuminating, he himself is guilty — as many historians of science are — of a fault of which he has accused Popper: that of giving too much weight to great historical transformations of scientific thought and too little to what goes on in laboratories from day to day. I don't myself believe in anything quite as humdrum as 'normal science'. A scientist tends to construct his own paradigm in the form of the opinions he already holds and carries over from day to day: hypotheses favoured because they have the right kind of 'feel', for

example, or through pride of possession of an idea which one was the first to have. But even this personal and most immediate paradigm is not clung to with the fervour of religious belief: I don't hold from one day to the next exactly the same opinion about any problem I am studying. A scientist's reading, discussions with colleagues and reflection on his latest experimental finding may change the climate of theoretical expectations from day to day. Little revolutions or movements of unrest are constantly in progress, and the real world of science is a kind of Maoist microcosm of continuing revolution. Kuhn, I think, envisages ordinary scientific life as something more in the nature of a settled opinion in a world of tranquil God-fearing middle-class contentment, and some of his opinions give me the impression of being held *pour épater le bourgeois*.

I do not want to represent this book as if it were in any way a pitched battle between the views of Kuhn and Popper. The comparison keeps on being made because Popper doth bestride this particular world like a Colossus. But forgetting all about comparisons, Kuhn's book is full of good things, with all the qualities to be expected in writing of a learned and highly intelligent man discoursing upon subjects of great interest and importance to scientists. I should also add that, considered as a disputant, Kuhn has the unmistakable address of a man who, so far from wanting to score points is anxious above all else to get at the truth of matters. □

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Newtonian pageant

Newton and Newtoniana, 1672–1975: A Bibliography. By Peter and Ruth Wallis. Pp. 362. (Dawson: Folkestone, UK, 1977.) £30.

THIS massive bibliography of writings by and concerning Isaac Newton is overwhelming in magnitude. There are 324 pages with an average of some 15–16 entries per page. This comes to more

than 5,000 books and articles, as some single entries are multi-volumed series. For the scholar, here is a pageant of the influence of Isaac Newton over three centuries, presented in a bibliographical memorial that embraces works in more languages than any single individual (historian or scientist) could ever read.

Four major kinds of works are listed by Peter and Ruth Wallis. First, there are

the writings of Newton himself, including the original printings, later editions, translations and excerpts, and collected works of various sorts. A second category includes articles, textbooks and monographs that either discuss Newton's scientific work specifically or are concerned with what could be called 'Newtonian science' in general.

A third category contains scholarly writings about Newton, such as biographies, analyses of a particular aspect of his method, a study of one of his books, an examination of his influence, and so on. This category also includes full-length biographies and criticisms or philosophical analyses of some aspect of his views on such topics as space and time, or his method in mathematics. In the seventeenth and eighteenth centuries, such works were part of the living tradition of science, concerned with the degree to which Newton's ideas should be accepted, modified, or rejected; but more recent articles in this category would treat Newton as a historical figure, or use Newtonian concepts or Newtonian science as a means of discussing a philosophy of science. Finally, there is a category of general works, such as encyclopaedias or dictionaries, which may discuss Newton's life and influence, Newtonian ideas, or even Newtonian science.

This bibliography has been compiled with meticulous attention to detail, often giving bibliographical minutiae that would be of little interest to the general scholar or scientist. For example, if the editors happen to know that there has been a reprint or offprint of an article in a journal, this gives rise to a separate entry in the bibliography, apart from the entry for the journal publication. It is difficult to know what value this information might possibly have, especially as almost all articles in scientific journals also exist in the form of offprints or reprints. Nowhere is the reader told what the criteria for inclusion were for articles, monographs and books. For example, there is an entry for Ramsey's *Newtonian Potential Theory*, presumably because the word "Newtonian" occurs in the title, but every work on this subject, and every book on dynamics or on general mechanics or on celestial mechanics could also have been included.

Some works of more primary importance for the study of Newton do not occur. One such is the *Lectures de Mécanique* of E. Jouguet, in which there is an important discussion of "les principes newtoniens". Only a few general histories of physics are included, but every book on the history of physics has a discussion of Newton. And the same is true with respect to chemistry and astronomy. Here a notable omission is the chapter in J. R. Partington's four-volume *History of Chemistry*, which is a mine of useful information and important insight. And it is the same with respect to physical

treatises on optics in the nineteenth and twentieth centuries, some of which deal extensively with historical materials; an obvious omission is the two-volume work the history of optics by Emil Wilde.

For some unaccountable reason, an attempt has been made to keep the major categories established in the old *Bibliography of the Works of Sir Isaac Newton* by George J. Gray, so that editions of Newton's mathematical writings, or historical and critical discussions of Newton's mathematics, have to be classified under either "Fluxions", "Arithmetica Universalis" or "Minor Mathematical Works". Accordingly, if a scholar turns to this bibliography as a guide to the literature on fluxions, he or she would not find any of the soundest and most illuminating writings on this subject, by D. T. Whiteside, whose annotated edition of Newton's fluxional writings is not to be found under any of the mathematical sections, but under "Collected Works, Correspondence, Bibliography". And a general article by Whiteside on the beginning of Newton's mathematical career lands up among "Minor Mathematical Works". It is difficult to know why there could not have been a section devoted to general historical and critical works on Newton's mathematics; and the same is true with

respect to Newton's physics, astronomy and philosophy. In short, although this volume is replete with all sorts of information, that information is not always complete, nor is it presented in such a way as to make it most useful for working scholars.

The above criticisms refer primarily to the works of scholarship of the past hundred years, rather than to the listings and descriptions of the writings of Isaac Newton himself and their various editions, reprints, translations, and presentation in excerpts, and the commentaries on them in the century or so after Newton lived. This portion is remarkably complete, and will serve as a primary guide for anyone who wishes to get information concerning any of the forms in which Newton's writings have been printed. Anyone who is interested in any aspect of Newton will be more than ordinarily grateful to Peter and Ruth Wallis for having prepared this bibliographical record. I doubt whether there is any Newton scholar, however learned, who will not find in these lists some references to works of real significance that will have hitherto escaped his or her attention.

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Oceanic micropalaeontology

Oceanic Micropalaeontology. Vols 1 and 2. Edited by A. T. S. Ramsay. Pp. 1453. (Academic: London and New York, 1977.) Vol. 1: £42.50, \$87; vol. 2: £36, \$70.25.

THE past decade has seen an enormous expansion in oceanic micropalaeontology, made necessary and possible by the unprecedented opportunities provided by the grand scale of the Deep-Sea Drilling Project (DSDP), and other oceanographic investigations. It is not always overtly acknowledged how many of the more startling recent advances in the Earth sciences (oceanfloor spreading, depth back-tracking, plate stratigraphy, ocean evolution, isotope and magnetic reversal stratigraphy, palaeoclimatology—including the results of the CLIMAP programme) are all fundamentally dependent on oceanic micropalaeontology in their application and verification.

Many of the results of relevant micropalaeontological investigations have appeared in recent years in the standard geological and palaeontological journals, and some new journals have been created to handle the flood of information. Much more material is enshrined in the massive series of Initial Reports of the DSDP. So many results have been obtained, in fact,

that it might have been thought helpful to bring together some of the more interesting in a review volume. This is not, however, what the present two-volume work on oceanic micropalaeontology does.

It is rather more a partial updating of the pre-DSDP volume on *The Micropalaeontology of Oceans* published by Cambridge University Press in 1971. Internal (and very occasionally explicit) evidence suggests that the articles were mainly written between 1972 and 1974, although the article contributed by the editor himself incorporates data that was not generally available until mid-1975; and there is evidence elsewhere in the volumes of revisions up to 1976. All in all therefore the contributions must have originated over the four-year period from 1972–76.

Volume 1 is concerned with planktonic Foraminifera and Pteropoda, 432 out of its 808 pages by B. A. Masters, comprising a massive catalogue of Mesozoic planktonic Foraminifera, and another 96 pages by W. A. Berggren being a partial catalogue of Palaeogene planktonic Foraminifera. There are two very useful reviews of the ecology and global distribution of modern planktonic Foraminifera and euthecosomatous Pteropoda by A. Bé, and Bé and R. W. Gilmer, which take up a further 175 pages. In the remaining 104 pages, W. F. Ruddiman considers the application of planktonic

Foraminifera to Quaternary climate in an article drawing extensively on work previously published elsewhere by himself and A. McIntyre. And W. N. Orr and D. G. Jenkins review planktonic Foraminiferal zonation schemes for the Caenozoic, partly in the light of selective solution problems. Throughout the volume the figures, whether line drawings or half-tones, are poorly reproduced; "excellent scanning electron micrographs" have been printed too dark to allow discrimination of detail, and print densities on other line drawings and half-tones are very variable. The plates associated with the article on Mesozoic Foraminifera are very wasteful of space.

Volume 2 contains a greater variety of contributions. Recent Caenozoic and Mesozoic Radiolaria are well reviewed in 142 pages by R. E. Casey, W. R. Riedel and A. Sanfilippo, and E. A. Pessagno. Recent and fossil calcareous nannoplankton are looked at from various points of view in 250 pages. Interesting aspects of the present biogeography and solution of the skeletal remains of coccoliths are dealt with by S. Honjo, K. R. Geitzenauer *et al.* and N. Schneidermann respectively, and W. W. Hay gives a very useful overall summary review of the stratigraphical development of the calcareous nannoplankton. Dinocysts, probably best known from marine epicontinental sediments, and the Silicoflagellates, a rather limited siliceous group, are reviewed by G. L. Williams and E. Martini respectively in a total of 114 pages. The remainder of the volume is given over to a discussion of automated biostratigraphy by T. R. Worsley and M. L. Jorgens, an account of the application of ^{18}O analysis to Quaternary microfossils by J. van Donk, and a review by the editor himself of the palaeoceanographical interpretation of Caenozoic deep-sea sedimentation. Of these three articles the one on automated biostratigraphy raises some interesting philosophical points about what is being attempted in the process of palaeontological time correlation, without producing conviction that the proposed methods will ever become widely adopted. Van Donk's summary is by now only one of a number on this important topic available to the interested reader in other readily accessible journals or serial publications. Ramsay's own contribution systematically applies pictographic analysis of the results of the DSDP to the end of leg 40 in an endeavour to interpret variations in chemical and physical properties in the oceans during the Caenozoic. Most of his arguments depend on analogies and methods (back-tracking, hiatus analysis, CCD determination) developed by a number of previous authors, and a lack of discussion of the fundamental processes at work makes it difficult to evaluate satisfactorily the validity of the circulation patterns inferred.

It is difficult to know how to make an overall summary judgement of such an expensive book. At this price very few individuals, and only a handful of specialist institutions, are likely to buy it. Yet several of the contributions it contains have clearly involved a real labour of love in their preparation, and as such deserve consideration by a much wider audience than they are likely to get. It is a pity that incorporation of large and less widely interesting contributions has at least doubled and probably trebled the length and cost of copies of the total work. The situation is not helped by the poor quality of reproduction of the illustrations throughout, by rather numerous spelling and printing errors, and by patches of undisciplined grammar that make parts of some articles literally incomprehensible.

Heavy rare earth metals

The Electronic Structure of Rare-Earth Metals and Alloys: The Magnetic Heavy Rare-Earths. By B. Coqblin. Pp. 656. (Academic: New York, London and San Francisco, 1977.) £29.50; \$57.75.

A MAJOR contribution to the literature on the rare earth metals, this book provides an extensive and detailed survey of those properties of the heavy rare earth metals from gadolinium to thulium which arise from the unique magnetic structures found in these metals. In 650 pages, and with over 680 references to the original literature, Professor Coqblin not only summarises the experimental data but also presents accounts of the underlying theoretical aspects in some detail.

The first two chapters present a preliminary survey of the experimental data and basic theory relating to the magnetic properties of the heavy rare earth metals. Chapters 3 and 4 take up the detailed theoretical description from the point of view of molecular field theory and spin-wave theory, including in each case some anisotropy contribution but not at this stage including magnetoelastic effects. Chapter 5 returns to experimental data and presents in detail the results relevant to the spin-wave theory discussed in chapter 4. In chapter 6 the wave vector dependence of the exchange integral is discussed in relation to the Fermi surface. The modification of the Fermi surface arising in the helical magnetic phases, which give rise to superzones and provide an explanation for the temperature variation of the turn angle, is also explained. Magnetoelastic effects are surveyed in detail in chapter 7, in which the author has

Oceanic micropalaeontology seems to be going through something of a publication crisis at the moment. An explosion of results is not finding altogether adequate means of expression, and seminal articles are appearing scattered through a wide literature. Several attempts have been made in recent years to bring together results in this field. None of them have been really successful and some have failed altogether. This contribution will be of some help to the interested reader seeking an overview, if he can get hold of a copy and if he is prepared to live with its transparent deficiencies.

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principally reviewed the role of magnetoelastic effects in driving magnetic phase transitions. It is in chapter 8 that the contribution of magnetoelastic effects to the spin-wave energy is set out in some detail in relation to neutron diffraction and magnetic resonance experiments. The final chapter presents a brief survey of band structure effects and transport properties.

There are two other books currently available to the research worker interested in the heavy rare earth metals which attempt to cover the whole field in some detail: *Physics of Rare Earth Solids* (Chapman and Hall: London, 1972) by K. N. R. Taylor and M. I. Darby is a wide-ranging review written at graduate level; *Magnetic Properties of Rare Earth Metals* (Plenum: New York, 1972) edited by R. J. Elliott, presents a series of monographs written by experts on particular aspects of the field. Coqblin's book provides a unified survey of the literature at advanced graduate level and complements the two other books. It will be welcomed by all research workers in the field and by those who have need to enquire about particular properties of the rare earth metals. However, those who do have need to use the book for specific reference should be warned that there is no survey of the crystallographical structures and metallography of the heavy rare earth metals and, much more regrettably, there is no survey of nuclear hyperfine interactions. Professor Coqblin has promised a further volume covering the other rare earth metals. We shall await this additional contribution with considerable confidence.

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Monitoring a nuclear test-ban treaty

Monitoring Underground Nuclear Explosions. By Ola Dahlgren and Hans Israelson. Pp. 440. (Elsevier: Amsterdam, Oxford and New York, 1977.) \$49; Dfl. 120.

DAHLGREN and Israelson have attempted to summarise the technical aspects of monitoring a comprehensive nuclear test-ban treaty, on a level that will be understandable to politicians and laymen, as well as to technical experts. The authors are seismologists who work for the Swedish government, which directs a large research program on nuclear monitoring and which has apparently encouraged them to produce this volume. This is a welcome effort, because technical disagreements in this area have often obstructed progress in arms-control negotiations.

The technical material in the book is drawn mainly from seismology, with small sections on nuclear explosion physics and on non-seismological means of identifying explosions. In addition, short non-technical sections review the history and current status of disarmament negotiations and discuss the political requirements for the monitoring of a comprehensive test-ban treaty (international data exchange and the like). Appendices give the texts of existing treaties related to nuclear explosions and list all known or presumed nuclear explosions that have occurred since 1963.

Non-technically oriented readers may find the book too long (355 pages of text) and too detailed for easy assimilation, largely because of the authors' attempt to impartially and exhaustively treat all aspects of the subject. A more selective approach would probably have been more useful to the non-specialist. The reports of the Stockholm International Peace Research Institute (*Seismic Methods for Monitoring Underground Explosions*, 1968, and 1971 *Progress Report*), which, though several years old, are not seriously obsolete, or Bolt's recent book (*Nuclear Explosions and Earthquakes*, Freeman, 1976) better serve the needs of laymen. Technically oriented readers, on the other hand, may find the book's detail useful. Virtually all relevant recent seismological studies are discussed, on a non-technical level. For those desiring more specific details, the extensive 26-page bibliography can guide them to the original sources, many of which are not in the open literature.

The book would be more useful if it avoided speculation in areas far re-

moved from seismology. For example, on p41 it states that "... we therefore think that it is unrealistic to imagine that one single person can build such a bomb" (referring to terrorists). On p338 the authors "express the strong feeling that nuclear weapons could be designed to maintain their long-term reliability without testing." On p340: "The 'military significance' of a test would generally increase with the yield of the explosion." On p50: "A series of tests is probably required to develop a militarily significant nuclear-weapon system." On p47, it is stated that typical test yields have decreased because of increases in delivery-system accuracy. These are all plausible guesses, but

only weapon designers know for sure. Such speculation lessens the book's authority.

It is unfortunate that such an expensive book has not been more carefully edited; virtually every paragraph suffers from defects of grammar or style (which do not, however, seriously affect the book's intelligibility).

All in all, the authors have produced a useful book, although they have probably fallen short of their ambitious goal of satisfying a highly varied audience.

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Physiological basis of sexual behaviour

Biological Determinants of Sexual Behaviour. Edited by J. B. Hutchison. Pp. 822. (Wiley: New York, San Francisco and London, 1978.) £19; \$37.

IN recent years, research into the physiological basis of sexual behaviour has advanced rapidly. This review volume therefore appears at a most opportune time and should particularly interest behavioural endocrinologists. Most of the chapters deal with well established laboratory species, especially rodents, and only a few authors, such as Kelley, Pfaff and Keverne, attempt to make their reviews truly comparative. However, this is partly a reflection of current knowledge, and is not intended as a criticism of the book.

The first of four parts deals with developmental processes, covering genetic and experiential factors as well as the influence of hormones on sexual differentiation of the brain. Part 2 is the outstanding section, comprising eleven chapters on various aspects of the neuroendocrine control of behaviour in adult vertebrates. All the chapters are well written and blend together successfully, despite a certain degree of overlap and repetition in some cases. For instance, much of the evidence concerning the "aromatization hypothesis" or the control of the lordosis response is repeated in several chapters. The effects of androgens on the hypothalamic regulation of sexual behaviour in males are discussed by Hutchinson, with particular reference to his extensive work on doves. Spinal mechanisms also make an important contribution to the patterning of masculine sexual behaviour, and Hart provides excellent coverage of this subject. The remaining chapters deal in the main with

hormones and sexual behaviour in female rodents, rhesus monkeys and humans. A crucial point referred to by some authors, and in particular by Herbert, is that gonadal hormones may exert their central effects on sexual behaviour by influencing monoamine neurotransmission. Meyerson and Malmnas provide a superb review of the psychopharmacological evidence, and Everitt describes a neuroanatomical approach to the study of monoamines, which promises to produce some important advances in future.

The conceptual framework and methods of measurement used in studies of sexual behaviour have changed considerably over the years and this improvement is likely to continue. Part 3 attempts to deal with these topics but it is disappointing. The two chapters are unequal to the task and do not enhance the more physiologically based reviews in parts 1 and 2. This section could, with advantage, have been much longer.

The final portion of the book is concerned with sociosexual behaviour. The individual contributions are excellent and cover a wide range of subjects including evolutionary constraints on patterns of sexual behaviour, the role played by olfactory and tactile stimuli, and mechanisms of "pair bonding" in avian species and of gender identity in humans. The authors in this section use widely differing writing styles but this does not detract from enjoyment of the material.

This is a very fine book indeed. Research workers should find it useful as it represents the most up-to-date review available on the physiological control of sexual behaviour.

Alan Dixon

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